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The price of trouble: £300

SHOULD university students be offered differing sizes of government grants depending on the subject they choose to take and its perceived relevance to national needs? The question has come into sharp focus in the United Kingdom with persistent rumours that the Department of Industry is trying to get engineering students paid an extra £300 on their annual grant. At present most students with state support get nominally identical grants, though some who receive grants from industry or the armed forces and who may have commitments after leaving university receive higher sums. Differential grants have been used in the past—in the 1930s potential teachers were given a supplement as undergraduates.

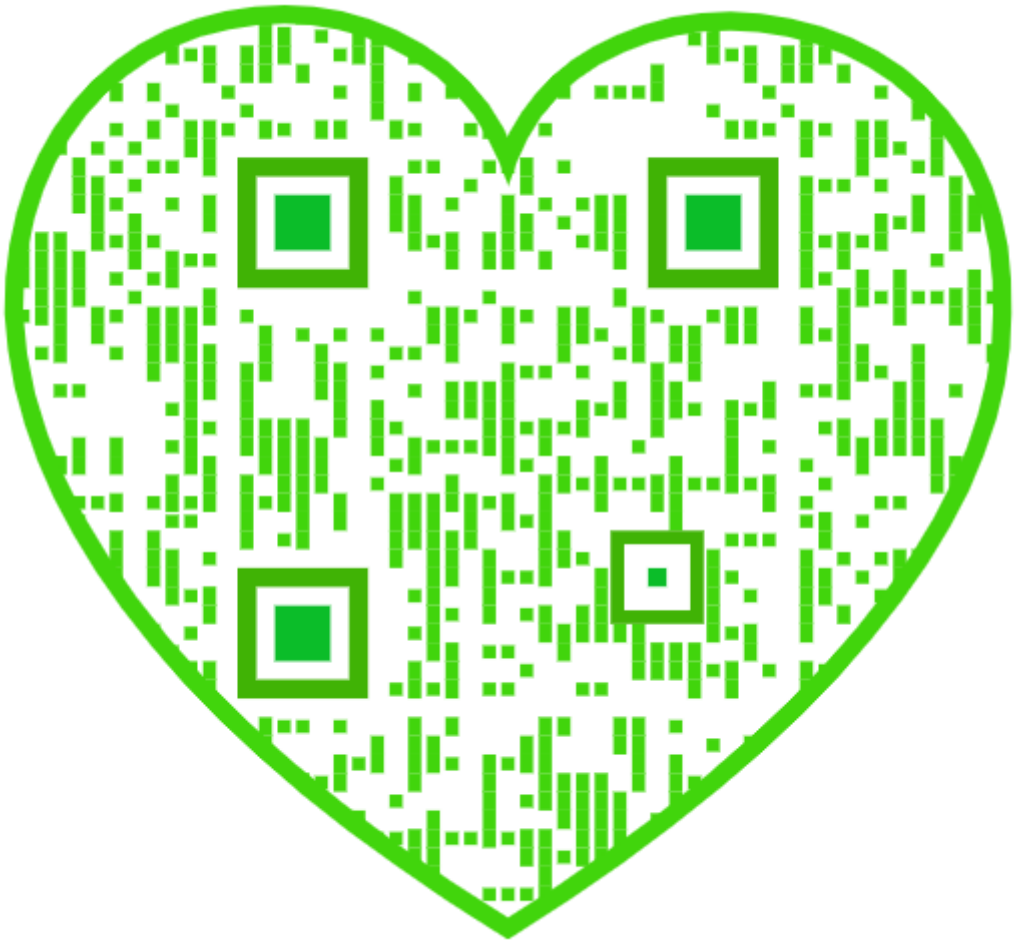
There are three questions which must be asked of any such scheme—is it needed; will it be effective; will it generate unnecessary trouble? There could be a need for differential grants if engineering graduates were in high demand, if universities had room for students and yet they were unforthcoming in sufficient quality and quantity. Obviously a simple label 'engineer' is inadequate for determining who is in high demand, and equally obviously many businesses which say they don't need more graduates are deceiving themselves. Even so, the supply of graduate engineers, whatever its defects, does not seem to have rung too many alarm bells. Nor, contrary to public opinion, are university engineering departments lonely places to be in these days. In 1976 there are 36% more students starting civil, mechanical and electrical engineering courses than there were in 1973 (during the same period the entry into mathematics, physics and chemistry dropped by 4%). The school academic attainments of engineering undergraduates are not conspicuously good, but it is by no means clear that £300 will buy quality; which brings us to the question of effectiveness of the measure.

If the measure turns out to be effective, it could easily be so by diverting students from adjacent disciplines: to collect £300 mathematicians would become mechanical engineers; physicists, electrical engineers; chemists, chemical engineers. Some effectiveness! Further, the measure can only be effective if schools are capable of responding to changing demands and are capable of giving wise advice to their pupils on whether the money justifies the switch in individual cases—yet many believe it is in schools where counselling on engineering careers is inadequately done. And the sort of student who will be tempted by £300 is also likely to look at the way engineering salaries compare with those in other professions—and maybe to decide that how the Department of Industry and how industry itself rates him are two different things.

Finally, the troubles it will cause. If engineering, why not science, for technology uses research results. Why not economics, because it is an economic mess we are in. Why not business management, because our companies need to be run better. Why not agriculture, because food imports make a big hole in our balance of payments. Why not design, which sells commodities. Or art, music, drama, which bring in tourists. The idea once accepted in one instance could lead to an almost endless queue of special cases. And once the scheme is started, how is it stopped without undesirable effects? After all, when ICI cut its graduate recruitment of chemists one year the consequences reverberated for years in schools and universities. So when the government phases out its supplement there can be no doubt that it will depress engineering recruits.

So, for a variety of reasons, the £300 scheme seems to be all wrong. But that should not deflect us from a deep concern that engineering does need some sort of leg-up from its present low-prestige intellectually-unsatisfying status. One thing about which the Department of Indsutry should be talking seriously to the Department of Education and Science is whether schools can provide adequate support and encouragement to potential engineers. A second is whether too many universities don't impose rigid barriers between mathematics, science and engineering thereby preventing students from 'tasting' engineering without making an irrevocable commitment.

It is not clear that opposition to differential grants at undergraduate levels should necessarily mean opposition at the postgraduate level, where the sacrifice of an industrial salary for the spartan life of a higher degree may for many engineers be intolerable in a way that it is not for most other research students who lack outside attractions. □



A view from home

Andrew M. Sessler, Director of the Lawrence Berkeley Laboratory (LBL) at the University of California, Berkeley, responds here to the assessment of LBL carried by Nature *(August 12, page 528)*

THE article on LBL was, in my opinion, neither fair nor accurate. I acknowledge the right of a writer to put down what he believes he has observed, but I cannot in good conscience say that I recognise our Laboratory either in its past or present state. In describing its past, for example, the writer, Wil Lepkowski, quotes Robert Yaes on the failures of Lawrence's Laboratory to discover artificial radioactivity and nuclear fission. Those are true charges, but they should surely be considered against a background of the many revolutionary discoveries which were made in the 1930s when Lawrence's Laboratory led the world in nuclear science.

Many fundamental features of nuclear reactions, nuclear structure, radioactive decay, nuclear chemistry and artificial radioactivity were established. The Laboratory was the birthplace of the transuranium elements, and has active research currently in progress to search for possible superheavy elements. The Laboratory established the science of nuclear medicine before the Second World War, when most of the radioactive tracers employed in medicine today were discovered and the foundations were set for the tracer techniques and scintillation detector cameras that are now so commonly used in diagnostic medicine. LBL's eight Nobel Prizes in fact reflect its vitality in basic science throughout Lawrence's time.

Mr Lepkowski is correct in his point that LBL no longer dominates the world of high energy physics, but the sense of failure he attaches to this seems unwarranted. It would be absurd to expect that any laboratory could continue to dominate such a thriving field. Yet our high energy physicists from LBL are not in fact doing all that badly. When the *Nature* article appeared the high energy physics community throughout the world was excitedly discussing the first observation of a charmed particle by a long-standing Lawrence Berkeley Laboratory-Stanford Linear Accelerator collaborative group. This same collaboration had made a major discovery two years earlier of the ψ/J particle, at the same time that the same particle was discovered at Brookhaven, and subsequently they discovered the ψ'. Furthermore, the collaboration of LBL and SLAC is a joint project in every sense of the word: they have been working together on this project for more than five years, using a national facility supported by the entire high energy physics community in the United States. LBL is proud to be an essential part of its design, construction and management, and can expect to continue to have a significant role in high energy physics research for decades to come.

Mr Lepkowski overlooks the strong and unique position that LBL occupies in heavy-ion nuclear physics. Our Laboratory has major programmes at its sector-focusing 88-inch cyclotron, the SuperHILAC and the Bevalac, the latter being the only source of relativistic heavy ions in the world. LBL accelerators are operated as national facilities and there are strong in-house experimental and theoretical groups. We are not blind to the fact that formidable competition is growing, particularly in Europe which is now allocating far more support to nuclear physics than is the United States, but at this time LBL is still considered by all to be a major international centre for nuclear physics.

Basic research is also very healthy at LBL in fields too numerous to catalogue in detail. Fundamental studies in biology and medicine work range from yeast genetics, physiology, and lipoprotein studies to radiobiology and nuclear medicine. LBL, in collaboration with others, is currently pioneering clinical trials on the efficiency of relativistic-energy heavy ions in cancer therapy. In molecular science we have some of the world's forefront studies on reactions in crossed molecular beams, on laser induced chemistry, and on quantum electrodynamic studies in high-Z hydrogen-like atoms.

In material science surface polaritons and plasmons are being studied by means of their nonlinear excitation through optical mixing of laser beams; and synchroton radiation is being employed, along with low energy electron diffraction (LEED), to ascertain the nature of catalytic surfaces. The fundamental understanding of alloys by use of electron microscopy and other advanced techniques has led to the ability to design steels and alloys of unprecedented toughness and hardness. From the field of astronomy there is the first measurment of the mass of a neutron star. In sum, basic science at LBL is diverse, excellent and enthusiastically pursued.

Mr Lepkowski dwells negatively on changes going on in LBL to address changing national and societal needs and a new set of goals from its major funding agency, the US Energy Research and Development Administration. Adaptation to a changing world is a necessity for organisations as it is for individuals. Furthermore, it is simply false to state that the old leaders and, in particular, the Nobelists, are still the dominating leaders of the Laboratory. In fact the Director, the Deputy Director, and eight of the ten Associate Directors have held their positions for less than four years. We of the present management make no apologies for changing the character at LBL.

We can also point to numerous entries into new fields in which important contributions have already been made and which have exciting prospects for the future. Among these are geoscience, geothermal resource assessment, controlled thermonuclear power, metallurgy, catalysis, isotope separation, materials science, environmental and biological effects of energy technology pollutants, energy policy analysis, solar power, and energy conservation studies. In our experience, when we enter fields which are non-traditional for LBL the quality of our scientific staff is revealed by immediate innovative use of advanced experimental techniques and theoretical analysis which result in important or revolutionary contributions to the new fields.

I can give the example of photoelectron spectroscopy turned to the study of the role of power plant particulates in the catalytic conversion of SO_2 to (harmful) sulphates; or the development, by a scientist previously working on the Lamb Shift, of the most sensitive detector for heavy metals by Isotope Shifted Zeeman Atomic Absorption (IZAA); or the development of superconducting quantum interference devices (SQUID) to a sensitivity some orders of magnitude greater than that available elsewhere, and their direct application to geothermal resource exploration. Or I could cite our work, some years ago, which initiated concern over the catalytic destruction of the protective ozone layer by supersonic transports.

I could give several examples, but let it suffice to say that we feel comfortable in our new activities because we know we are doing excellent science and engineering on problems of importance to mankind. □

Exotic viruses

Arie Zuckerman offers this brief on some of the diseases now attracting broad attention

A BRIEF perusal of the daily press over the past few months would reveal an incidence of unusual disease which, to many, seems quite remarkable. There have, most recently, been the illnesses reported from the Sudan, Nigeria, Zaire and Sierra Leone—now apparently identified, by the World Health Organisation last week, as Marburg disease. Work on the identification of Philadelphia's so-called Legion's Disease continues in the United States. Lassa fever meanwhile attracts considerable attention. Rabies and Swine influenza receive even greater emphasis. Even Bubonic Plague has been in the headlines.

Of the many viruses that can infect more than one species of animal, three are of particular current interest. Rabies, with a huge reservoir of infection in the wildlife population of most parts of the world, is now racing across Europe towards Britain, where it has not been endemic for more than half a century. Marburg disease, first identified nine years ago, is still not fully understood. And Lassa fever looms as an awesome viral infection in tropical Africa.

Marburg disease

Marburg virus (or "green monkey") disease, a severe, distinctive, febrile human illness, was first described in 1967. Thirty-one cases of illness with seven deaths in Germany and Yugoslavia were traced to direct contact with blood, organs or tissue cell cultures from a batch of African green monkeys caught in Uganda. Several secondary cases subsequently occurred in hospital personnel who had been in contact with blood from patients; one further case was apparently transmitted by sexual intercourse. Although 29% of the primary cases proved fatal, there were no deaths in the 6 secondary cases.

It soon became apparent that this was a previously unrecognised disease, caused by an infectious agent probably new to medical science. Laboratory work using guinea pigs and Rhesus and vervet monkeys revealed an elongated sinuous organism generally curled in the form of a figure 6 or a horseshoe with superficial resemblance to rabies virus. But Marburg virus is morphologically distinct and does not share any antigenic properties with rhabdoviruses or with any other known viruses.

The infection has been investigated intensively since the first outbreak, but the natural cycle of transmission in nonhuman primates and perhaps other species of animals and the origin of the infection remain unknown. The disease has not hitherto been recognised in a sporadic form in Africa.

The first recognised outbreak of the disease in Africa, and the first since the original outbreak, occurred in South Africa in February, 1975. The victim, a young Australian man who had hitch-hiked through Rhodesia, died in a Johannesburg hospital; shortly afterwards his travelling companion and one of the nurses who had cared for him contracted the same disease. Both recovered. Virological investigations showed the outbreak was caused by the Marburg virus. As in the original outbreak in Marburg, when the virus was isolated from semen 83 days after the onset of illness evidence was obtained that the virus could persist in the body for at least 2–3 months after the initial attack; the virus in this case was isolated from the victim's eye fluid 80 days after the onset of the illness.

Lassa fever

The dramatic story of Lassa fever began when two missionary nurses from Lassa, in North-Eastern Nigeria, died in 1969 from a mysterious illness; a third, who was gravely ill and flown to the United States, recovered. Convalescent plasma from her was effective in the treatment of a laboratory worker, who acquired the infection while working with tissue cultures infected with blood from these patients.

The following year a further outbreak of Lassa fever killed 52% of 23 patients admitted to hospital in Nigeria. Dr Jeannette Troup, who was primarily responsible for drawing attention to this disease, also died of the infection. Since then further cases have occurred and 10 out of 21 medical workers have died from Lassa fever. Up to June, 1975 the disease was recognised on nine different occasions involving 114 officially recorded cases, two of which resulted from laboratory infections. Over a third of these infections were acquired by person-to-person spread within hospitals. Many other clinically suspect cases have since been uncovered from reviews of hospital records: retrospective searches through the medical literature have revealed references to cases closely resembling Lassa fever in the Central African Republic, Upper Volta, Nigeria and Sierra Leone. Since 1969, cases have occurred in Nigeria, Sierra Leone and Liberia, and serological evidence suggests the virus may have been active elsewhere in West and Central Africa. And of course recent cases identified in air travellers to Britain, the United States and Canada have received extensive press coverage.

The clinical spectrum of Lassa fever extends from very mild or inapparent illness to fulminating fatal infection. Among patients admitted to hospital the death rate is as high as 67%, with more fatalities among Caucasians than Asians. Intimate contact with any primary case appears to carry a high risk of infection; relatives or medical attendants providing direct personal or nursing care are particularly threatened. Cases have occurred among patients and hospital visitors who apparently had no direct contact with the primary cases. Only a few such tertiary infections have occurred, and the reasons for the interruption of

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Keystone

Lassa patient arrives in Hamburg from Nigeria

outbreaks after the generation of secondary cases are not clear. It seems that, although the primary cases were highly infectious, the secondary cases were not.

Lassa virus is a member of the arenavirus genus, a group of enveloped RNA viruses with a virion of granules instead of a defined core. Along with Machupo virus (which causes Bolivian haemorrhagic fever) it produces a significant mortality in man. Lassa appears to have the greatest potential for direct spreading in human populations. It is related antigenically to the rodent-associated haemorrhagic viruses of South America and to lymphocytic choriomeningitis virus, a persistent infection of mice. It has been isolated from a single rodent species, the multimammate rat *Mastomys natalensis* in West Africa.

More recently, a virus closely related immunologically to Lassa virus was recovered from the tissues and organs of wild *Mastomys natalensis* captured in 1972 in Mozambique. This finding more than doubles the potential area of Lassa-like virus distribution in Africa. The multimammate rat is the most widespread and commonest rodent in Africa south of the Sahara, and an ideal carrier to the domestic environment because of its semi-commensal habit and a combination of other factors, including an exceptionally high propagation rate. Whether all the *Mastomys* rodents are capable of maintaining and transmitting Lassa virus, is not known, and the ecological interrelationships between *Mastomys* and the presumed non-reservoir genera *Rattus* and *Mus* have yet to be determined. Carnivores, in particular dogs and cats which probably prey upon *Mastomys* and other rodents, have not yet been investigated as potential secondary hosts.

The Lassa virus may spill over from the rodent cycle to man by various routes including, for example, contamination with rodent urine, directly or on foodstuffs or through dust. The means by which the virus is spread from one person to another is not clear. Accidental inoculation with a sharp instrument or contact with blood has accounted for a few cases. The virus has been isolated from the pharynx and urine of patients, so that indirect airborne spread of the virus as well as mechanical transmission are most likely.

Lassa fever is not a disease which comes under international quarantine regulations, which at present offer the only effective means of reducing the risk of transmission. Transmission can be limited to some extent by surveillance, identification, and the hospitalisation and isolation of patients, but quarantine of households may have to be considered by public health authorities, and ways of reducing rodent populations should be explored. The evacuation of patients presents special problems. Until effective methods of treatment and control become available and a suitable vaccine is developed, the epidemiological propensities of this infection may be very wide with the modern means of air travel.

Rabies

Rabies virus is a member of the rhabdovirus group, which includes a diverse collection of characteristic bullet-shaped RNA viruses from mammals, reptiles, fish, insects and plants. The concept of a single antigenic entity is no longer accepted since there are four recognised rabies-related viruses, although these appear to be confined to sub-Saharan Africa. Two of these viruses, Mokola and Duvenhage, are associated with fatal rabies-like human illness.

Rabies is a viral infection of the central nervous system which has been recorded in most domesticated and wild warm-blooded animals. The virus, present in salivary secretions, is transmitted mainly by bites or scratches, although the inhalation of minute droplets in the air can also lead to infection, and oral transmission has been demonstrated in experimental animals. The susceptibility of an animal depends on its age, the degree of infection, the site of introduction and the properties of the particular virus strain involved. Once the virus leaves the site of inoculation it usually passes to the central nervous system through the peripheral nerves. Once the central nervous system has become infected the virus may multiply, attacking such tissues as the salivary and other glands, the kidneys, the brown fat, and, in some species, muscle and lung. In some infected organs the virus is found primarily within the nerves.

The effects of the disease on the nervous system and the change of behaviour in the victim are probably important to the mode of transmission. Typically, the sick animal (and this applies particularly to small carnivores) becomes unusually and indiscriminately aggresive, while in the earlier stages of infection timid animals become less restrained, approachable and friendly. High population density and turnover and long incubation periods maintain the infection even though it is usually fatal. Man is irrelevant to survival of the virus: there is no adequately documented case of human transmission of rabies, although a theoretical risk exists because the virus is present in secretions.

The most important pool of infection in most areas of the world is the wildlife population, including, in particular, foxes. Examination of thousands of small wild rodents in the Americas has not revealed rabies infection, but in parts of Europe the isolation of rabies virus with unusually low virulence has been reported from wild rodents. The significance of this finding is not clear, and cannot be immediately associated with fox rabies. The ecology of bat rabies is intriguing: in the Americas, numerous species of bats have been found with rabies infections, but in many areas these infections are independent of other mammalian cycles. The disease is readily passed on to cattle by the bite of vampire bats during feeding; in Latin America, for example, bat rabies is estimated to have killed many hundreds of thousands of cattle. Over 170 human deaths from rabies have been attributed to vampire bats, which suck the blood of sleeping victims. But natural transmission from insectiverous bats to other terrestrial animals by biting has not been observed, and experimental transmission with infectious saliva has proved difficult.

The major problem caused by rabies, according to a paper from the Office of Health Economics in the UK, does not stem from its immediate effects, but from the medical, social and economic risks and the cost associated with its prevention. Improved human diploid cell vaccines are in fact now becoming available for pre- and post-exposure prophylaxis in man, while supplies of human rabies immunoglobulin for passive immunisation and local wound treatment are expected to increase and developments in intensive care promise a better outlook for future victims.

The control of rabies in animals, on the other hand, presents considerable difficulties. The international transfer of animals must be rigidly controlled, and strict precautions should be taken in the acquisition of mammals from the wild. The control of wildlife rabies is even more difficult. Hormonal sterilisation can be effective experimentally but has not yet been used successfully in the field. The immunisation of wildlife may become feasible in the future, particularly oral immunisation of foxes using bait doped with live attenuated virus. The control of bat rabies requires further investigation, although inoculation of cattle with small amounts of anticoagulant has proved lethal for vampires. Similarly the backs of captured vampire bats can be smeared with anticoagulant mixed with petroleum jelly: their release has resulted in dramatic reduction in the number of bats, since the treated bats contaminated others in their colony by contact. □

NUCLEAR TRADE

More words than action

International trade in nuclear technology was a key issue at the 20th annual meeting of the International Atomic Energy Agency (IAEA) held in Rio de Janeiro last month. Bruce Handler reports from Brazil

ONE reason the IAEA meeting was held in Latin America was to encourage a better understanding of the problems facing developing countries in the field of nuclear energy. Nearly 1,400 delegates who attended wound up hearing almost as much about problems IAEA members have with each other as they have with nuclear energy, because a good portion of the debate consisted of political tirades. But everyone was in favour of the further development of nuclear energy. The sharp divisions were over which countries should assume the responsibility for developing it, and to what extent.

Many industrialised nations expressed fears over putting sophisticated nuclear technology into the hands of underdeveloped Third World countries, principally because of the widely shared belief that such countries could be encouraged or provoked into diverting this knowhow for military purposes. A corollary fear was that terrorists could stage a raid on an underdeveloped country that possessed nuclear reactors and steal the necessary components for making an atom bomb, even if the country in which this occurred was actually using atomic energy only for peaceful purposes.

The Third World rebuttal of this position was that underdeveloped countries could never progress if they are not allowed to acquire modern technology, in nuclear energy as well as in other fields. Developing countries described as discrimination the desire on the part of industrialised nations to limit the spread of such technology.

The IAEA's director-general, Sigvard Eklund, noted that many Third World countries have not signed the 1970 Nuclear Non-Proliferation Treaty because of this feeling. He appealed to them to reconsider the political reasons which originally led to their taking such a position but later recognised, realistically, that when it comes to national pride, nations sometimes refuse to listen to the most soberly reasoned arguments.

The country most strongly opposed to the sharing of nuclear technology was the United States. The head of the US delegation, Robert C. Seamans Jr., director of the US Energy Research and Development Administration, said in an address that the USA did not want to see sophisticated atomic knowhow spread throughout the developing world.

He said that countries which are taught how to enrich their own uranium for use in nuclear reactors could use this knowhow to separate plutonium from uranium. This, in turn, Seamans stated, "can increase the risk of diversion (of fissionable material) to nuclear weapons and also the risk of terrorist activities." The United States was willing to enrich uranium for other countries and make it widely available, but it was a risk to international security to show developing nations how to do their own enriching. Seamans added that as a compromise, the IAEA could set up regional uranium-enriching centres, which would at least be under multinational control.

Yugoslavia presented a proposal for creating a multinational pool of nuclear resources, to make it easier for developing countries to begin or expand nuclear energy programmes.

The IAEA meeting, however, was basically a forum, rather than a policy-making session, and the best any single delegate could hope for was that others would consider the proposals discussed and take them back to their home governments for study and possible implementation.

The Third World view on technology sharing was expressed most emphatically by Hervasio de Carvalho, the president of Brazil's Nuclear Energy Commission. He labelled the preoccupation over safeguards and the spread of nuclear technology to underdeveloped nations as "exaggerated", and repeated what Brazilian government officials have been trying to

One complete session of the IAEA meeting was devoted to the scientific discussion of advances that have been made in the use of nuclear technology in food and agriculture. Among the highlights:

- Dr Maurice I. Fried, of the United States, director of the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture, gave a historical introduction to the use of nuclear techniques in the field of food and agriculture, and told delegates that isotope and radiation techniques "are neither esoteric nor so sophisticated that developing countries cannot apply them for the solution of practical problems." He noted that the bulk of the work of the joint FAO/IAEA programme he heads is aimed at helping developing countries use atomic energy to improve their agricultural output.
- Dr Dick De Zeeuw, of the Netherlands, general director of agricultural research of the Netherlands Ministry of Agriculture and Fisheries, offered the sombre forecast that by the year 2010 the world will have 1,800 million more people who consume less than the required daily amount of food than it does today, and that most of these new hunger victims will be in Southern and Southeast Asia. Many developing countries, he said, now lose from 20–40% of their crops after harvest; food-irradiation and related techniques could reduce this loss, but the main obstacle was the failure of policy-makers in developing countries to adopt the widespread use of atomic irradiation of food and to discard ineffective traditional methods.
- Dr K. Sundaram, of India, director of the biomedical group of the Bhabha Atomic Research Center in Bombay, made the point that effective food preservation techniques are difficult to transfer to underdeveloped ones, because of problems of money, materials, technology and energy of the poor nations' end. He said that developing nations around the world had failed to recognise irradiation's true importance, and criticised them for wrongly copying food preservation methods from developed countries, which, he noted, do not need to place so much importance on irradiation because of the already high standards of their food industries.
- Admar Cervellini, of Brazil, professor and head of the Physics and Meteorology Department of the University of Sao Paulo's Luiz de Queiroz Agricultural College, in Piracicaba, spoke about the work of Brazil's Centre for Nuclear Energy in Agriculture, of which he also is the head. The centre, which was set up with IAEA assistance, is now ten years old.

Dr Cervellini said that one of the centre's most practical achievements has been the development, through radiation mutation, of strains of beans with increased yield, with higher protein content, and with more resistance to Golden Mosaic disease. Seeds of the latter variety have been distributed to five other Latin American countries.

explain to the rest of the world since the controversial nuclear deal with West Germany in 1975: that Brazil, which is poor in oil, has low-grade coal and is quickly using up its feasibly exploitable hydroelectric potential, has no other alternative but atomic energy for meeting its future electric-power needs. And for reasons of national security and common sense, Brazil cannot remain dependent on outside suppliers for nuclear fuel—as the United States would like.

Carvalho pointed out that even though Brazil has not signed the Nuclear Nonproliferation Treaty (for the usual reason: opposition to the division of the world into an elite, industrialised "atomic club" and everyone else), it has submitted the agreement with West Germany to IAEA safeguard supervision. This, he said, is proof that Brazil is not going to make any bombs, adding that the world should accept the word of other countries that make similar pledges to use atomic energy only for peaceful purposes. (The IAEA Board of Governors, which met in Rio before the general meeting, approved safeguard provisions in two other major transfer-of-technology nuclear agreements: the one between Canada and Spain and that between France and South Africa.)

The Nigerian delegate's contention, that developing countries are in the IAEA "to be seen but not heard" and that they are "merely tolerated" by industrialised countries, did not seem to be reflected by the vote on a proposal sponsored by Iraq to admit the Palestine Liberation Organisation to the IAEA, as an observer. Of the 71 of the 110 member countries of the agency that were present for the vote, 46 voted in favour, 21 abstained and only four (Israel, the United States, South Africa and Costa Rica) voted no.

A move led by black African countries and supported by several socialist states to expel South Africa from the IAEA, on grounds that the Pretoria government does not represent the people, did not succeed. The best these countries could do was to get a motion passed to review South Africa's permanent seat on the Board of Governors, but with no action to be taken until the 1977 IAEA meeting. It is unlikely that even this watered-down proposal will bring results, because IAEA by-laws say the permanent regional seats on the board must go to the countries in each specific area of the world that are the most advanced in nuclear technology. In Africa, there is no doubt that this is South Africa.

The effect of nuclear power stations on the environment was another topic to come up at the meeting. Eklund expressed veiled dislike for citizens' movements which have sprung up in countries such as the United States to stop further construction of nuclear reactors. The IAEA chief asserted that nuclear power plants are "friends of the environment." He said there has never been a fatal accident from nuclear causes at any of the 180 non-military nuclear installations now operating around the world. In contrast, Eklund noted, there have been serious accidents at hydroelectric plants, including the bursting of dams and the consequent widespread destruction of the environment.

Debate continued at the meeting on how best to dispose of waste material from nuclear reactors, by burying it in the ground, storing it in protected containers, dispersing it into the air or dumping it into the ocean. Perhaps predictably, no consensus was reached.

USA

New directions in lobbying

A new lobbying organisation concerned with global scientific environmental and social issues was established in the United States last week. Colin Norman reports from Washington

ALTHOUGH Washington is thick with lobbyists, public interest groups, research organisations and assorted political hucksters selling commodities ranging from the B-1 bomber to environmental awareness, the debut last week of a new 'citizens lobby' deserves —and is getting—more than passing attention.

Called 'New Directions', the organisation will be devoted to raising Washington's political consciousness (or at least, the consciousness of Washington's politicians) on such international issues as arms control, population growth, environmental pollution, and the depletion of resources. It will be an unabashed lobbying enterprise, focusing its attentions on the Congress. The reason it is already attracting considerable notice is that the list of its officers and governing board reads like a *Who's Who* of people concerned with such matters.

Full-time President of New Directions is Dr Russell W. Peterson, former Governor of Delaware, who recently resigned as Chairman of the Council on Environmental Quality to head the new group. And the outfit's executive council will be chaired by Dr Margaret Mead, the well-known anthropologist who has long been active in public interest causes associated with science.

Planning for New Directions began in the Summer of 1974, when Norman Cousins, editor of *Saturday Review*, and Theodore M. Hesburgh, president of the University of Notre Dame, convened a small group to discuss whether such an organisation is needed and if so, what form it should take. During the past two years, the planning group has expanded to more than 100 people, including such luminaries as Robert McNamara, head of the World Bank, Kingman Brewster, President of Yale University, James Grant, president of the Overseas Development Council, Lester Brown, head of the Worldwatch Institute, and Sargent Shriver, former director of the Peace Corps.

The organisation will be similar in structure to Common Cause, a lobbying group which concentrates its attentions on domestic affairs. It will be a grass-roots organisation, financed by membership fees of $25 per year, solicitations for which will soon be made through a direct mail campaign using membership lists of sympathetic organisations. New Directions hopes to attract at least 300,000 members, a number which would give it a very sizable war chest to put its message across.

Although the general nature of that message is evident from the concerns of the people associated with New Directions, priorities and details of the organisation's objectives are now being mapped out by groups of individuals working in the general areas of arms control, poverty and the widening economic gap between rich and poor nations, environment and natural resources, human rights, and governmental decision-making.

There are already numerous organisations active in Washington in those areas, but New Directions hopes to provide an additional function—political lobbying to get the concerns raised by various public interest organisations through to the policy makers. Few existing organisations have been able to take on such a role because they would lose considerable tax advantages if they were to indulge in direct lobbying activities. Essentially, they are registered as educational or research outfits. Though a recent change in the tax laws has lifted some of those restrictions, few organisations are geared up to lobby actively.

A statement put out last week by the planning committee of New Directions

describes its relationship with existing groups. "While the work of these groups provides an essential base of information and documentation," it says, "the missing element has been a force unlimited in scope and unfettered by tax prohibitions . . . No organisation has set out to mobilise existing resources to take hard political action on the critical measures which must be implemented to relieve the problems which now disrupt all economies, undermine political stability, suppress human freedoms, and directly threaten human survival throughout the world. Our purpose is to build such an organisation".

The idea is that New Directions will work closely with other groups—indeed, many of them have representatives on the organisation's governing board—and draw heavily on their research and expertise. It will select issues to lobby for, try to bring together informal coalitions of groups to work on them, and serve as a coordinating centre for exchange of relevant information. But its chief role will be to draw as much attention as possible to global problems and their potential solutions, through direct lobbying with members of Congress, government officials, corporate executives and so on. It will also take its case to the news media, organise local groups, go to court when necessary, and provide direct support to candidates for political offices.

Clearly, although the organisation will derive much of its influence from its star-studded cast of backers and officials, the crucial factor in its effectiveness will be the size of its membership. Politicians tend to listen more attentively to messages delivered by groups backed by large numbers of voters or by large amounts of cash which can be used for campaign support. If New Directions can succeed in attracting hundreds of thousands of members, its voice will be heard above the general background noise in Washington.

At this point, however, it is difficult to predict whether or not the organisation can count on that level of support. The people most likely to join are probably already members of existing groups such as Common Cause, and it remains to be seen whether the depth of their concerns extends to spending another $25. But there is at least one promising indication: the planning committee decided to go ahead only after a survey by a New York market research firm found considerable support for the goals of the organisation.

Another potential problem for the organisation is that unless it treads carefully, it may end up stepping on the toes of existing bodies which are already concerned with global problems—such as the United Nations Association, the Overseas Development Council and the Worldwatch Institute. A check with people from some existing groups last week, however, found little fear that New Directions would steal their thunder or interfere with their goals. In fact, most welcomed the possibility of having a heavyweight group fighting for their causes.

Some supporters of New Directions also argued last week that one of the organisation's chief assets is the fact that it is headed by Russell Peterson. A PhD chemist who worked for DuPont for 26 years, Peterson is a Republican who built a good reputation during his term as Governor of Delaware between 1969 and 1973. Peterson also knows his way around the Washington power structure very well, having served as chairman of the President's Council on Environmental Quality for three years. During that time, he took several independent stands, the latest of which was to call for immediate regulation of fluorocarbons in aerosol sprays. As one observer put it last week, a Republican, a former governor, and a former top adviser to the President are good credentials for a lobbyist. □

FDA's aerosol ban

In a statement which caught most observers here completely by surprise, the Food and Drug Administration (FDA) announced last week that it intends to phase out the use of chlorofluoromethanes (so-called fluorocarbons) as propellants in many types of aerosol sprays. Although the statement did not say when the ban would take effect, or how it would be implemented, FDA said that it would begin by requiring warning labels to be placed on aerosols containing fluorocarbons, and that the ban would be imposed in an "orderly" manner.

The agency's action is surprising since it follows hard on the heels of a major report by a committee of the National Academy of Sciences which recommended that regulation of fluorocarbons should be delayed for up to two years to allow time for more research on the mechanism by which fluorocarbons are believed to be breaking down the ozone layer. The academy report suggested that since the rate of destruction of the ozone layer is small, a two-year delay in regulation would present little additional hazard.

Dr Alexander M. Schmidt, the Commissioner of FDA, said last week, however, that additional research would narrow the range of uncertainty in the calculations of ozone depletion but it "won't change the ultimate regulatory situation". He argued that "given the effects on human health even a 2% ozone depletion from 'unessential' uses of fluorocarbons is undesirable".

"The known fact", Schmidt said, "is that fluorocarbon propellants primarily used to dispense cosmetics are breaking down the ozone layer. Without remedy, the result could be profound adverse impact on our weather and on the incidence of skin cancer in people. It's a simple case of negligible benefit measured against possible catastrophic risk".

FDA's authority extends over foods, drugs and cosmetics. Thus, in theory, it has the power to regulate the formulation of products such as hair sprays and anti-perspirants which account for the bulk of aerosol uses of fluorocarbons. According to FDA's statement, the agency has authority over about 80% of all products now packaged in aerosol containers.

Schmidt said that FDA will publish details of its proposal to require labelling of aerosols containing fluorocarbons in mid-November, and that details of the phase-out programme will follow a few weeks later. The labelling programme itself should reduce consumption of fluorocarbon-containing products, and it will also help discourage stockpiling.

A spokesman for the DuPont company, the chief manufacturer of fluorocarbons, said last week that he was "astounded" by FDA's decision. The industry had regarded the academy's report as a victory because, in recommending a two-year delay in regulation, the academy had essentially backed the industry's argument that more research is needed to settle scientific uncertainties.

The industry is unlikely to take FDA's action lying down. Although industry spokesmen would not say last week how they expect to contest the ban, it is likely that they will ask for public hearings and, if necessary, go to court to prevent FDA putting its proposals into effect. One possible legal challenge may involve whether or not FDA has the authority under existing laws to take such action. The intensity of the industry's reaction will depend largely on how much time FDA gives it to phase out use of fluorocarbons, however, and that won't be known until towards the end of the year.

USA

● The International Council of Scientific Unions (ICSU) is the latest organisation to seek a role for itself in the mounting debate over the risks and benefits associated with recombinant DNA experiments. At its 16th General Assembly, held in Washington last week, ICSU decided to establish a Committee on Genetic Experimentation to undertake a variety of tasks, ranging from the provision of advice and information to anyone who seeks it, to the support of lectures and training courses on safety techniques. The committee will also look into the possibility of providing ICSU support for a facility where large populations of cloned fragments of, for example, mouse or human DNA will be constructed and maintained for use by individual scientists. One aim of the committee is to use ICSU's considerable influence to try to ensure that the various controls on recombinant DNA experiments which are being imposed by countries around the world are consistent with each other. As Sir John Kendrew, ICSU's secretary general put it last week, it doesn't make much sense for one country to put very strict controls on the rsearch and for another to allow experiments to go ahead with few restrictions.

But Kendrew acknowledged that the countries with which ICSU deals "are sovereign states and you can't tell them what to do". The committee (which has the snappy acronym COGENE) will thus work behind the scenes, collecting and distributing information and providing assistance to national committees where it can.

Part of COGENE's task will be to ensure that recombinant DNA experiments are allowed to proceed, albeit under "appropriate and generally agreed safeguards". Dr William J. Whelan, who headed a committee which wrote COGENE's charter, noted last week, for example, that "the committee is not coming into being to preside over the demise of genetic manipulation and to that extent, ICSU is expressing a position".

The committee's most valuable function, however, may well be its provision of training courses and other technical assistance for recombinant DNA researchers. Kendrew pointed out that in smaller countries training in biological safety techniques may be limited, and ICSU could provide a needed service. The committee will also look into ways to aid in the distribution of strains of crippled hosts and vectors for recombinant DNA work, to help ensure that such strains are universally available.

As for the suggestion that COGENE should provide assistance for a central clone bank for fragments of mammalian DNA, the establishment of such a facility could greatly reduce the proliferation of some of the more hazardous cloning experiments. A similar clone bank is under consideration in the United States, for use by American scientists.

● Once again, the Food and Drug Administration (FDA) has refused to allow the artificial sweetener cyclamate back on the market in the United States because of unanswered questions about its safety. Cyclamates were banned by FDA in 1969 on the basis of studies which indicated that they may cause bladder cancer in test animals when fed to them in large quantities; Abbott Laboratories, the manufacturer of the sweetener, contested the ruling and has been trying ever since to get it reversed. Last year, Abbott presented FDA with a petition, backed by a sheaf of test data, purporting to show that cyclamates are safe. But, last week, after turning Abbott's svidence over to an independent committee for its opinion, FDA rejected the petition, stating that "the data submitted . . . do not establish that cyclamate acid, calcium cyclamate, and sodium cyclamate are safe for their intended use". Undeterred, Abbott has demanded a public hearing, and will probably press its case in court if necessary. The final curtain has still not dropped on the longest-running food additive farce in Washington.

● The Presidential election campaign drones on, with scarcely any mention of scientific, environmental or related issues (the only exception being some discussion of ways to curb nuclear proliferation). But at least the candidates' thoughts on three matters of interest to the scientific community have appeared in print.

The current issue of *Physics Today*, a magazine published by the American Physics Society, contains the responses of President Ford and Jimmy Carter to three questions put to them by the APS president, William Fowler. Fowler asked for their views on the role of science advisers in the White House, on national energy needs and the nuclear power programme, and on federal support for basic and applied science.

On the role of scientists in White House policy-making, Ford referred to the fact that Congress has recently approved legislation he introduced to re-establish a White House Office of Science and Technology Policy (OSTP). Noting that the director of the office will be the President's science adviser, Ford stated that he would "be one of my senior advisers and will also provide advice to other senior advisers". Carter offered no details of the role of his science adviser, though he argued that "the office of science adviser to the President should be upgraded immediately to provide a permanent and high-level relationship between the White House and the scientific community".

The two candidates differed most in their replies on energy policy. Ford contended that the use of both coal and nuclear power should be expanded, called the safety record of nuclear plants "outstanding", said he had increased federal spending on safety technology, and noted that his Administration was then in the middle of a review of its nuclear policy.

Carter said that no leadership now existed in the White House on energy planning. He said strong emphasis should be placed on energy conservation, arguing that "50% of our energy is wasted". On nuclear power, Carter stated that "we must make every effort to minimise our dependence on nuclear energy", though he emphasised that he does not support a nuclear moratorium. He said he would shift research priorities from nuclear power to conservation and non-nuclear options.

On federal research support, Ford said he had increased budgets for basic research and criticised Congress for reducing his budget proposals for the National Science Foundation. Carter noted that the federal government "has a crucial role to play in supporting development of new technologies which address national priorities".

Colin Norman

USSR

● The aborted Soyuz 23 mission, although failing to accomplish any of its scientific objectives, has nevertheless won itself a place in the history of Soviet space flight by being the first major mission publicly acknowledged to have failed.

Hitherto, there has been a consistent policy for disguising failure, malfunction of lunar or interplanetary probes being disguised as part of the cover-all Kosmos programme (Kosmos 96 and 111, for example, were almost certainly originally intended as Venus and lunar probes respectively); a manned mission which returned to earth prematurely was explained in the media as having "successfully carried out its objectives". If, as in the case of the nightlanding of Soyuz 15, early return involved some special hazard, it was then claimed that the testing of recovery techniques under these difficult conditions was itself part of the programme. Even the tragic conclusion of the first Salyut–Soyuz mission, when the returning cosmonauts perished on re-entry, was offset by official pronouncements that all experiments had been successfully concluded and that scientifically speaking the mission had been entirely successful.

This reticence regarding setbacks was not lifted to any great extent in the preparatory work for the joint Apollo–Soyuz flight, for which the Soviet planners supplied information on a "need-to-know" basis, and there was relatively little discussion of past missions. Exchange of scientific information is a major concept of detente; the effective acknowledgement of difficulties both in the mission of Soyuz 23 to the Salyut 5 spacelab and in its return and landing may prove to be a sign of greater openness on the part of the Soviet space planners.

● The Far Eastern Science Centre of the Soviet Academy of Sciences is failing to make a proper practical contribution to the national economy. That, at least, is the finding of a special enquiry by the People's Monitoring Committee of the USSR. Over the past five years the Institutes of the Centre have carried out research on "more than 400 topics of a theoretical and practical character", notably in geology, vulcanology and marine resources. But, says the report, there were serious "derelictions" in the institutes' work which have prevented the proper utilisation of scientific potential and resources.

Too little is being done to apply the results of completed research to the national economy; during the last Five Year Plan, only 20 projects "with a confirmed economic effect" were realised, and out of 142 proposed inventions, only 42 received patents. The majority of research resulted merely in publications and "unplanned-for" monographs. The Centre is therefore deemed to be failing to carry out one of its fundamental functions, that of coordinating research "within the framework of the regions".

The development of the local resources and potentialities of Siberia and the Soviet Far East was the main reason for the founding of local Filials of the All-Union Academy, and the combining of existing Institutes into "Science Centres". Thus the Buryat Filial of the Siberian Branch of the Academy of Sciences of the USSR, which has recently celebrated its tenth anniversary, concentrates on solid state physics, rare-metal chemistry, and polymer synthesis—all in the name of local development—as well as the "propagation of electromagnetic waves in the conditions of the Trans-Baikal area", which, it is claimed, has brought television to more than 80% of the inhabitants of Buryatia.

The criticism of the Far Eastern Science Centre—in contrast to a practically-oriented body such as the Buryat Filial—is fully in accordance with the directives of the current Five Year Plan, which, like its predecessor, stresses the concept that research should serve the economy. In many cases, however, this demand is little more than a slogan, invoked in the reportage of all major projects, even when, as in the case of an interplanetary probe, the practical benefit to the economy seems somewhat tenuous.

As Mr Brezhnev reiterated in justification of basic research at the 25th Party Congress, "There is nothing more practical than a good theory". The findings of the investigation on the Far Eastern Science Centre suggest, however, that, at least in the lower echelons of the scientific establishment, a greater emphasis on immediate practical applications may from now on be obligatory.

● Construction of the first full-scale geothermal power station, at the foot of a large volcano on the Kamchatka peninsula, is about to begin. Kamchatka and the Kuriles form the only region of active vulcanism in the USSR: during the past year eight new volcanoes have appeared on the Kamchatka peninsula, one of which is continually active, while on Matua Island in the Kuriles an eruption of the Sarycheva volcano began on September 23, emitting a column of lava bombs, ash and steam, with explosions every 1½–2 minutes and an ash cloud so dense that vulcanologists could not approach the island for eight days.

The possibility of using geothermal waters has long been considered a promising energy source for the development of local mineralogical and fish-processing industries. A pilot generator, at Pauzhetka, has been in operation for some time. According to information presented at a recent All-Union Symposium held in Kamchatka and the Kuriles on the utilisation of geothermal heat-sources, "There are quite sufficient high-temperature resources for geothermal stations with a total capacity of 300 MW".

● The pilot U-25 MHD-generator, which came into service in 1971, and which still remains the only one of its kind in the world, is to receive a new superconducting magnetic system, developed at the Argonne National Laboratory in the USA. In exchange, American scientists will have an opportunity of carrying out research on the U-25 jointly with their Soviet colleagues. MHD power generation is one of the main fields of Soviet–US cooperation, and the supply of the new magnetic system will form a major step in the implementation of intergovernmental agreements. The Director of the High-Temperature Institute of the Soviet Academy of Sciences, Academician A. Sheindlin, says results from research on this generator are of particular significance because it has already been in operation for some 4,000 hours, for 200 of which it has been supplying electric current direct to the Moscow grid.

Vera Rich

CANADA

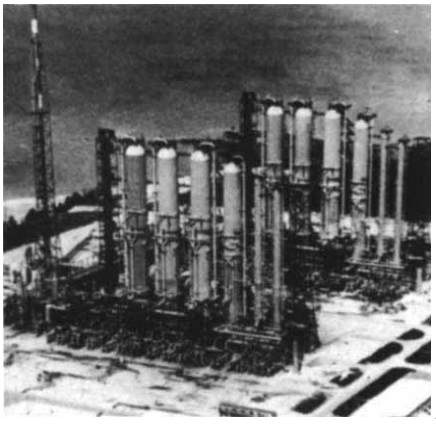
Rethinking nuclear policy

A contribution to Canada's nuclear debate has come from the head of the Science Council of Canada. David Spurgeon reports from Ottawa

THE executive director of the Science Council of Canada, John J. Shepherd, has called for the federal government to set up a concentrated, focused, nuclear industrial strategy. The call, his most recent public pronouncement, indicates how much he has done to shape a new, bolder, more public and more independent role for the Council, something it has sought over this past concluding year to its first decade.

Shepherd came to the council from industry, where he was chairman of a successful high technology instrument firm, and he brought with him the vigorous, pragmatic approach one would expect from such a background. So his public statements tend to be hard-hitting and to the point. This latest, which was contained in an article in *The Financial Post,* was no different.

Shepherd acknowledged that there are serious matters still to be resolved regarding the nuclear power issue in Canada—like other industrialised countries, Canada has become locked in debate over questions like the safety and security of reactors and the disposal of radioactive waste. But it stated that nuclear power in Canada "is a fact —and it would be foolish not to take advantage of the opportunities it offers." And it went on to point out that the size of the proposed nuclear power programme in Canada in future is very large: an estimate of 70 nuclear power units by the end of the century is decidedly conservative, which means the market will be an average of $1,500 million a year for the next 25 years.



AECL

Bruce heavy water plant, Ontario

This means, said Shepherd, that "it is imperative that we devote a good deal of attention to planning." Three sectors are involved in the Canadian nuclear programme: Atomic Energy of Canada Ltd (AECL), a Crown corporation that carries out research, development and engineering; the provincial electrical utilities, which operate the plants; and industry, which does the manufacturing. "It is painfully clear," said Shepherd, "that industry has so far been unable to carry its weight in this arrangement."

This comment was nothing new: as far back as the 1960s, AECL officials were saying the same thing. But Shepherd went on to say that industry's contribution has been rendered ineffective by cancellations or postponements in nuclear plant construction, lack of a steady stream of nuclear projects, low-volume ordering and low profit margins. Those wanting to break into the market, particularly in some of the specialised instrumentation areas, were frustrated by piecemeal orders. What is required, he added, is a "mixed nuclear consortium—comprising electrical utilities, AECL, and industry", and for this to happen, several changes would have to be made.

Electrical utilities would have to alter their construction philosophies and permit others to play a greater coordinating role. AECL would have to hand over to the new consortium its Power Projects group, which carries out its engineering functions. And industry would have to accept new responsibilities. If AECL's heavy water production activities were also transferred to such a consortium, it would leave only its original research and development function. Under another name this could become the institutional focal point for a major thrust in energy research development, "for example, along the lines of the US Energy Research and Development Administration. Such an expansion of responsibilities might also contribute to the initiation of a national energy policy."

Shepherd again made it clear he thought the domestic market for nuclear power station more important to Canada than foreign ones, and referred to losses associated with a nuclear sale to Argentina. Others have pointed to difficulties Canada has had with sales to countries like South Korea, India and Pakistan. If Canadian industry could not grasp the opportunities presented by the domestic nuclear market, "it should not complain when government fills the vacuum." □

IN BRIEF

Nobel prizes announced

The $160,000 Nobel prize for medicine will be shared by Professors Baruch Blumberg and Carleton Gajdusek. Dr Blumberg is professor of Medical Genetics at the Institute for Cancer Research of the University of Pennsylvania and Dr Gajdusek works at the National Institutes of Health, Bethesda; both men did their prize-winning work at the National Institutes of Health. Both prizes are for research in virology, Professor Blumberg's for discovering Australia Antigen, a particle associated with serum hepatitis (hepatitis B), and Professor Gajdusek's for the fundamental research on kuru, the slow virus disease that was prevalent in the cannibalistic Fore tribe of New Guinea.

The prize for physics goes to Professor Burton Richter of Stanford and Samuel Ting of MIT for their work on the J/ψ particle. The particle, discovered simultaneously and independently by the two researchers in 1974, has opened up new realms of investigation with the new property of matter known as 'charm'.

The prize for chemistry is awarded to Professor William Lipscomb of Harvard for his work on boranes. The bonding of these compounds was long a puzzle according to conventional valency ideas; Lipscomb in the 1950s took the new multicentred-bonding theory, predicted borane structures and used elegant X-ray crystallography to show that some of these structures were actually cages, one even an icosahedron.

2,4,5-T production ended

Britain's only producer of 2,4,5-trichlorophenol, the chemical being manufactured at Seveso when the poison TCDD was accidentally released, has decided not to recommence production. The company, Coalite and Chemical Products Ltd, of Bolsover, Derbyshire, stopped production in August "to make 110% sure" of its safety measures. Coalite had operated with more stringent safety measures than the Italian plant, but after the Seveso accident UK Health and Safety inspectors recommended even more precautions. The company has blamed over-sensational publicity of the Seveso accident for its decision.

● A list of 721 highly poisonous substances has been prepared by the Ministry for the Environment of the West German State of North Rhine Westphalia. Included are all chemicals with an effect similar to that of TCDD, some even more poisonous and some less poisonous but still potentially lethal.

Argentinian release

Reports coming out of Argentina indicate that most of the employees of the Atomic Energy Commission who have been in prison since April (*Nature*, October 7, page 452) have now been released. Dr T. Victoria, whose brother highlighted their problems, is now in Belgium; the rest are still in Buenos Aires. There is, however, still no news at all of Antonio Misetich, a one-time MIT researcher.

UK physicists' concern

The UK high energy physics community is becoming increasingly concerned at the prospect of financial setbacks to its research effort. This became clear last week with the emergence of attempts to organise its members for concerted action. These follow the Science Research Council's recent urgent request to large laboratories for information on possible early cutbacks in expenditure.

One possibility being canvassed is that there should be a letter-writing campaign to ministers, MPs and the Advisory Board of the Research Councils, which has pursued a deliberate policy of squeezing big science. Another is that the most distinguished members of the nuclear and high-energy physics community might be able to agree on some form of corporate action to defend their interests.

The government's cash limits doctrine, which with a depreciating pound strains the SRC's international obligations, has precipitated the crisis.

FBR decision postponed

Britain's decision whether to build a demonstration commercial fast breeder reactor, due this autumn, has been delayed to give more time for public debate. Mr Anthony Wedgwood Benn, the UK Energy Secretary, has also indicated that the questions put last week to the Nuclear Installations Inspectorate concerning the fast breeder are designed to assess its margins of safety independently for the public's benefit. France's Phénix prototype fast breeder at Marcoule closed down recently for a period of weeks because of a leak in one of its heat exchangers.

Ariel V's birthday

The UK's Appleton Laboratory celebrated the Ariel V satellite's second anniversary on October 15. Ariel V, launched off the coast of Kenya, is controlled from the Appleton Laboratory which rapidly transmits data to experimental groups at the universities of London (University College and Imperial College), Leicester and Birmingham; the Goddard Space Flight Center in Maryland also operates one of the experiments.

On responsibility

KENNETH MELLANBY

DOES science, or do scientists, have a special responsibility to society? A conference held early in October in Florence, Italy, and organised by the Fondazione Internazionale Menarini, considered this subject from a number of angles. Experts from many countries gave papers on the problems arising from genetic engineering, ecological contamination of the biosphere, world food shortages, safer drugs for better therapy, the specific needs of developing countries and population overgrowth. The speakers identified many fields in which scientific research and its application obviously have an important part to play. They suggested that the public and their rulers often underestimate the contributions that scientists may make. But the general conclusion seemed to be that though scientists should be more vocal about their possible value, they should generally advise their rulers and should not formulate policy. In fact, they should continue to be "on tap, not on top".

Most speakers stressed the importance of work aimed at solving practical problems of food and health, and the need of more support for such investigations. However, others suggested that the important and soluble problems might not be so easy to identify, and they justified their efforts in more basic fields. For instance, it was said (predictably, by scientists with world reputations in the subject) that we know so little about the processes going on in the oceans that all manner of apparently-academic studies are justified in the hope that we may, eventually, have the knowledge to control marine pollution. It was interesting to hear views so reminiscent of the "Haldane principle" on which government-supported research in Britain was based until its recent reorganisation.

I could not help wondering how personally responsible were the scientists who were discussing responsibility. I found it interesting to observe the behaviour of many of the participants at this meeting. They had been transported freely from the ends of the earth, to be lavishly entertained by their Italian hosts, not only to the best of the food and wine of the country, but also, between sessions, to the art and music of Florence. I regret to have to report that I have seldom attended a meeting at which such a substantial number of the delegates put in such a poor attendance. Many appeared to feel that so long as they read a twenty minute paper (replete, in many cases, with material familiar to their meagre audience) and listened to a few of the other speakers at the same session, they were free to slip off to attend the "ladies" sightseeing tours or to go shopping or indulge in other non-scientific activities. This hardly seemed an example of scientific responsibility; surely delegates attending a conference at someone else's expense should be prepared to attend most of the meetings and contribute to the proceedings?

So is doing good work in the laboratory and attending meetings conscientiously the sum total of the scientists' responsibility to society? Our Marxist colleagues do not think so; it was a relief at the Florence meeting to be spared their diatribes urging the adoption of political dogmas as the acme of scientific fulfilment. I do not think I am alone in my belief that scientists are responsible not only for their own work, but for trying to ensure that this work, and that of their colleagues, is effectively organised and applied. If we are right in our views that many recent developments in scientific organisation have been harmful to both science and to society, it is our responsibility to try to have a better system adopted. We should do this no matter how unpopular it may make us with some of those in authority, and even though, under the present system, we depend on those same authorities for both support and (in the case of our younger members) the furtherance of our careers.

correspondence

Not a MAFF gaff

SIR,—The attack by Kenneth Mellanby in your issue of September 16 (p186) quotes recent newspaper interpretations of reports by the Ministry of Agriculture, Fisheries and Food (MAFF) but does not quote from the reports themselves, which are a strictly factual account of our food supply. The MAFF evaluates Britain's food supply at two different levels. The first provides an assessment of the total food available for human consumption in the UK from home production and imports, after deductions have been made for exports and non-food uses; the second (the National Food Survey) provides an assessment of food bought at the retail level by different types of household. Professor Mellanby's article appears to be based on newspaper comments on the former, which was published in *Trade and Industry* on August 27 of this year (p596).

Value judgements are not appropriate to these published accounts, and the MAFF nowhere suggests in them that "Britons eat too little food of any kind", or that "the quality of food consumed shows a steady decline", or that "the consumption of more animal protein leads to better nourishment". Rather, it is clear that an average energy value of 12.16 MJ (2,910 kcalorie) per person per day is substantially more, not less, than any recommendation from the United Nations' Food and Agriculture Organisation. Furthermore, the most recent report of the UK National Food Survey Committee shows that the food purchases of British households provide substantially more of almost every major nutrient than is recommended by the UK Department of Health and Social Security, and that the nutritional quality (estimated as amounts of nutrients per 1,000 kcalorie) is in several respects increasing.

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Tobacco substitutes

SIR,—Tobacco substitutes cannot be so neatly classified as the article by Allan Piper implies (*Nature*, September 2, p2); nor is it relevant, as the article might also imply, to predict the toxicological characteristics of tobacco substitutes from smoke chemistry data.

Tobacco itself, of course, has both 'organic' and 'inorganic' components. Both NSM and Cytrel, which are currently the subjects of submissions to the UK Hunter Committee, are 'organic' to the extent that each contains cellulose derivatives as combustible fuels, and glycerol as a humectant; and both these tobacco supplements are also 'inorganic' to the extent that each contains a proportion of mineral fillers.

Both NSM and Cytrel produce smoke, the composition of which differs considerably from that of tobacco smoke; and to rely upon the chemical composition of smoke to predict toxicological characteristics, as Piper's article can imply, is unwise. The fact is, and there is ample evidence for this from many other aspects of toxicology, that our understanding of the biological effects of chemical compounds, acting alone or in combination, is quite insufficient to allow any such prediction to be made. Instead, reliance must be placed primarily upon a comparison with tobacco in terms of tests which provide direct measurements of biological activity relevant to diseases associated with cigarette smoking, and upon other toxicological tests to afford an acceptable assurance in respect of risks to health not associated with cigarette smoking. This need is very clearly recognised in the first report of the Independent Scientific Committee on Smoking and Health. The results of such direct comparisons using NSM have been most reassuring.

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Limiting parameters

SIR,—During the past decade or more, articles published in scientific journals have been increasingly disfigured by the use of the word 'parameters' to include any variables that can be measured (*Nature*, September 16, p387). This practice, though discouraged by one learned society (Medical Research Society), now seems to be blessed by the Shorter Oxford English Dictionary (OED)—(see Addenda p2649). The added meaning is "a distinguishing or defining characteristic or feature esp. one that may be measured or quantified, 1962" that is, a variable. The 1964 edition of the Shorter OED gives several meanings of the word 'parameter' specific to certain disciplines such as mathematics, astronomy and crystallography, but defines its general meaning as "a quantity which is constant (as distinct from the ordinary variables) in a particular case considered, but which varies in different cases: esp. a constant occurring in the equation of a curve or surface, by the variation of which the equation is made to represent a family of such curves or surfaces, 1852". This definition seems to me (with relics of schoolboy Greek to conform with *παραμετρεω, "to measure one thing by another, to compare, Plato"* (*An Intermediate Green–English Lexicon*, Liddell and Scott, 1955).

Oxford lexicographers may exculpate themselves with the defence that they merely record current usage in both spoken and written form (even though it tends to perpetuate a nonsense that a parameter may be sometimes a constant, at others a variable). But do editors of scientific publications, which require exactitude for permanent records, need to be so fickle? If the Editor of *Nature* were to insist that in his journal the word 'parameter' in the general sense shall have the 1852 meaning, would not editors of all journals less widely read soon follow suit, to the great benefit of all discerning readers?

This clarification of the current fog would not resolve the underlying difficulty that there is a persistent demand for a word to embrace the measurable and estimable quantities of a system, for which the word 'parameters' is now fashionably used. If a factor is measurable, there is little harm in referring to it as 'a measurable'; but if it is a derived number like the constants, a or b (parameters) of the equation of direct proportionality of the two variables x and y ($y=a+bx$) it is not directly but only indirectly 'a measurable'. In the collective sense measurables plus estimables are 'quantifiables' or 'quantities', or 'numerables'.

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The Editor of *Nature* welcomes all correspondence. Letters should be kept as brief as possible.

news and views

Molecular heterogeneity of the β^0 thalassaemias

from Edward J. Benz Jr

THE β thalassaemias are characterised by an inherited reduction (β^+ thalassaemia) or total absence (β^0 thalassaemia) of β-globin chain synthesis. There is general agreement that defects in β-chain synthesis occurring in β^+ thalassaemia are due to corresponding reductions in the amount of RNA coding for β chains (Nienhuis and Anderson, *J. clin. Invest.*, **50**, 2459; 1971; Benz and Forget, *J. clin. Invest.*, **50**, 2755; 1971; Housman *et al.*, *Proc. natn. Acad. Sci. USA*, **70**, 1809; 1973; Kacian *et al.*, *Proc. natn. Acad. Sci.* USA, **70**, 1886; 1973). But as indicated by the recent paper of Ramirez *et al.* (*Nature*, **263**, 471; 1976) the β^0 thalassaemias may be far more complex and heterogeneous with respect to their precise molecular origins.

Several genetically and clinically distinct haemoglobinopathies are associated with the absence of β-chain synthesis. The "typical" or "high A_2" type of β^0 thalassaemia represents the extreme form of β^+ thalassaemia; β-chain synthesis is totally absent and haemoglobin A_2 ($\alpha_2\delta_2$) levels are elevated. In both $\delta\beta$ thalassaemia and hereditary persistence of foetal haemoglobin (HPHF) δ and β-chain synthesis are totally absent. In HPHF the balance between alpha-chain and non-alpha-chain synthesis is preserved by efficient synthesis of the γ chains of haemoglobin F ($\alpha_2\gamma_2$). In $\delta\beta$ thalassaemia, γ-chain synthesis is insufficient to compensate for the absence of β-chain synthesis, resulting in a thalassaemia phenotype. Finally, Hb Lepore syndrome is characterised by absent δ and β-chain synthesis, incomplete compensation by γ-chain synthesis and production of small amounts of Hb Lepore ($\alpha_2[\delta\beta]_2$). The $\delta\beta$ chain is a fused chain consisting of the N-terminal regions of δ chains and the C-terminal regions of β chains. Hb Lepore is thought to arise by an unequal crossover event during meiosis, resulting in deletion of intact δ and β-chain genes and creation of a fused $\delta\beta$ locus.

Conconi *et al.* (*Nature*, **238**, 83; 1972) have suggested that the high A_2 form of β^0 thalassaemia is heterogeneous at the molecular level. In their studies, reticulocyte polyribosomes from patients with β^0 thalassaemia from the Ferrara Valley region of northern Italy synthesised no β-globin chains when incubated with postribosomal supernatant fractions prepared from the same Ferrara-type β^0-thalassaemia reticulocytes. But "induction" of some β-chain synthesis was reported when these polyribosomes were incubated with non-thalassaemic supernatant. Conconi *et al.* (*Nature*, **254**, 256; 1975) later reported data suggesting that intact reticulocytes from these patients synthesised some β globin after therapeutic blood transfusion. In contrast, non-thalassaemic postribosomal supernatant fractions did not induce β-chain synthesis by polyribosomes obtained from non-Ferrara β-thalassaemic reticulocytes (Rowley and Kosciolek, *Nature new Biol.*, **239**, 234; 1972). Conconi *et al.* proposed that the β mRNA in Ferrara thalassaemia was normal; the primary genetic defect was thought to be a deficiency of some factor essential for β-mRNA translation.

Direct studies of globin mRNA translation in heterologous cell-free protein-synthesising systems by Benz *et al.* (*Blood*, **45**, 1; 1975), Kan *et al.* (*Proc. natn. Acad. Sci. USA*, **72**, 5140; 1975), and Tolstoshev *et al.* (*Nature*, **259**, 95; 1976) have subsequently demonstrated absence of translatable β-chain mRNA in non-Ferrara forms of β^0 thalassaemia. Ramirez *et al.* have now translated globin mRNA isolated from Ferrara patients before, and 22 d after, blood transfusion. No synthesis of β globin was observed when these mRNA fractions were incubated in a cell-free system fully supplemented with all the factors necessary for efficient β-chain synthesis. All forms of β^0 thalassaemia studied directly thus seem to be characterised by absence of functional β mRNA.

Total absence of functional mRNA could result from absence of the β-chain globin gene (gene deletion), failure of the β-globin chain to function as a template for mRNA synthesis (transcription), or synthesis of structurally abnormal mRNA incapable of functioning in protein synthesis. Evidence favouring each of these mechanisms has now been reported. The paper of Ramirez *et al.* typifies the diversity of results obtained. Each laboratory studying this problem uses the same general methodological approach. Purified synthetic DNA copies (cDNAs) complementary to human α and β mRNA are isolated and used as molecular probes in cDNA–mRNA or cDNA–DNA hybridisation assays designed to quantify the relative amounts of α and β mRNA or DNA present in the sample being studied. Ramirez *et al.* have demonstrated that the β gene is present in high A_2 type β^0 thalassaemia but absent in HPHF, confirming recent work by others (Tolstoshev *et al.*, *op. cit.*; Ottolenghi, *et al.*, *Proc. natn. Acad. Sci. USA*, **72**, 2294; 1975; Kan *et al.*, *Nature*, **258**, 162; 1975; Forget *et al.*, *Cell*, **7**, 23; 1976). Ramirez *et al.* also report the important new finding that the β-chain gene is absent from the genome of patients with homozygous $\delta\beta$ thalassaemia. The authors cite unpublished results by Ottolenghi *et al.* (submitted for publication) from which the same conclusions were reached. There is thus general agreement that at least two genetic mechanisms account for different haemoglobinopathies associated with absent β-chain synthesis: gene deletion in $\delta\beta$ thalassaemia and HPHF, and the presence of a gene which does not code for active β mRNA in high A_2 type β^0 thalassaemia.

There is considerably less agreement about the lesion in β-mRNA metabolism responsible for absent β-chain synthesis in patients with high A_2 type β^0 thalassaemia. Forget *et al.* (*Nature*, **247**, 379; 1974) and Tolstoshev *et al.* (*op. cit.*) have reported virtual absence of β-chain mRNA in non-Ferrara patients with β^0 thalassaemia. In contrast, Kan *et al.* (*op. cit.*) studied two Chinese patients with β^0 thalassaemia and found nearly normal amounts of apparently intact β-chain mRNA. Ramirez *et al.* now report that reti-

culocyte RNA from Sicilian patients with β^0 thalassaemia contained significant amounts of β-mRNA which hybridised efficiently to the β-cDNA probe. The hybridisation behaviour of mRNA from patients with Ferrara-type thalassaemia was much more complex. Some "β-like" mRNA was detected, but this mRNA could protect only 40–50% of the β cDNA from digestion by the single stranded specific S_1 nuclease used in the hybridisation assay. The β-like mRNA being detected was either deficient in major portions of the sequence represented by β cDNA, or possessed a base sequence so grossly altered that very poorly matched hybrids were formed.

In summary, published data suggest that β^0 thalassaemia and related haemoglobinopathies can arise from lesions in virtually every step of globin mRNA metabolism: gene deletion (in HPHF and in β^0 thalassaemia), presence of the gene but total absence of β mRNA, presence of grossly abnormal β mRNA in Ferrara-type thalassaemia, and presence of mRNA with normal hybridisation properties but no template activity. Unfortunately, it is not clear at present whether the diversity of results reported reflects the true heterogeneity of these syndromes or, to some extent, methodological differences among the various laboratories investigating this problem. For example, Forget *et al*, and Kan *et al.* obtained very different results when they analysed the same mRNA sample from a Sicilian patient with β^0 thalassaemia. Forget *et al.* reported near absence of β mRNA while Kan *et al.* found 30–70% as much β mRNA as α mRNA. Therefore at least some of the differences reported may be due to technical factors rather than to any fundamental heterogeneity among the β^0 thalassaemias.

Many theoretical and practical considerations demand cautious interpretation of the data obtained from different laboratories. Reticulocyte counts tend to be rather low in β^0 thalassaemia and blood samples must often be transported from endemic areas to the laboratories equipped for hybridisation analysis. One is thus dealing with very small amounts of RNA, isolated, in many cases, from blood subjected to variable conditions of storage and transport. More importantly, the reticulocyte represents only the end stage of erythropoiesis; the mRNA levels measured in reticulocytes may not reflect accurately the true status of mRNA metabolism during erythropoiesis. For example, if all patients with a high A_2 type β^0 thalassaemia produced an unstable β-mRNA molecule, the different results obtained in different laboratories might merely reflect the "age" of the reticulocytes isolated at a given time from a given patient, contamination of reticulocytes by circulating nucleated red blood cells, variations from patient to patient in the degree of mRNA instability, or differential losses of α and β mRNA during storage of the sample. Since "β mRNA" content is in fact measured as the ratio of β/α-mRNA sequences, these factors could produce either over- or under estimates of β-mRNA content, depending on which message (α or β) was more severely affected.

Close homology exists among the amino acid sequences of β,γ (39/146 differences from β), and δ (10/146 differences) globin chains. Partial cross-reaction among the mRNA and cDNA sequences specific for these chains is known to occur unless incubation conditions are carefully designed to eliminate cross-hybridisation. In all the aforementioned studies the hybridisation conditions were designed to eliminate γ–β cross-hybridisation. It is, however, much more difficult to deal with the potential problem of δ–β cross-hybridisation. It has not yet been possible to isolate δ mRNA, estimate the "normal" amounts present, or determine whether δ–β cross-hybridisation occurs in "stringent" hybridisation conditions. Finally, some laboratories use cDNA probes which are incomplete copies of their mRNA templates or are significantly contaminated (10–30%) with other globin-specific cDNA sequences. Although such probes are useful for detecting the presence or absence of β mRNA, they are less reliable for quantifying the precise amounts present.

Although the above technical reservations are significant, there is little doubt that some molecular heterogeneity exists among different patients with β^0 thalassaemia. For example, the β mRNA detected by Kan *et al.* in reticulocytes from Chinese patients had thermal denaturation profiles (melting temperature curves) indistinguishable from those of normal β mRNA. These patients, at least, seem to have some true β mRNA. Similarly, Benz, Forget, and coworkers (*Clin. Res.*, **23**, 269a; 1975, and manuscript in preparation) have analysed mRNA from a number of patients with β^0 thalassaemia using highly purified full length cDNA probes. In some of these patients the absence of message could be demonstrated, while in others the presence of at least small amounts of β-like mRNA was observed. In these studies, a patient with Hb Lepore syndrome had mRNA which hybridised to the β probe, suggesting indirectly that the δ–β mRNA (and, presumably, δ mRNA) can cross-hybridise with β cDNA even in "stringent" conditions.

Precise definition of the genetic lesions in different patients with β^0 thalassaemia is important because each of these defects represents a potentially important clue for elucidation of the poorly understood pathways of mRNA synthesis, processing, transport and stability. Further progress will require detailed investigation of mRNA metabolism in nucleated erythroblast precursors. It is to be hoped that there will be free exchange of samples so that general agreement may be reached as to the final results obtained. It is crucial that the results obtained reflect only differences in the state of the β^0 thalassaemia genes rather than differences in the state of the art in various laboratories.

The thalassaemia syndromes have been unique among human disease states because of the facility with which the techniques and concepts of molecular biology can be applied to explain the genetic and biochemical origins of the clinical disorder. Further study of the β thalassaemias should continue to yield valuable information about the factors controlling the metabolism and stability of globin mRNA in normal erythroid cells. □

Changing climate of the North Atlantic

from P. M. Kelly

THE debate over the causes of climatic change is slowly shifting from lively, but somewhat unproductive, discussion of the theoretical likelihood that any climatic control, such as solar activity or ocean–atmosphere interaction, could affect climate, to discussion concerning the relative importance of selected controls that have been convincingly shown to have affected climate. This synthesis has been hampered by the reluctance of some investigators to examine the assumptions upon which their chosen method of analysis is based and to consider the extent to which their conclusions are determined by such assumptions. For example, the results of some numerical modelling experiments tend to stress the importance of internal fluctuations in the ocean–atmosphere system and to minimise the importance of external factors such as solar activity, simply because these external factors are assumed constant. Similar considerations apply to all investigators who concentrate on observational evidence related to one external factor alone. The causes of climatic change are complex and it is unlikely that any one approach to the problem, or any one climatic control, will provide a full answer. To assess

Molecular insight into thrombosis

from E. W. Horton

WHY don't platelets stick to the surface of healthy blood vessels whereas they will stick to foreign surfaces such as glass or to damaged or diseased blood vessels? The recent discoveries of two naturally occurring compounds have revealed much about the underlying chemical mechanisms and may provide an answer to this intriguing problem.

In 1975 Samuelsson and his group at the Karolinska Institute in Stockholm discovered that when platelets stick to each other (or aggregate) they release a substance called thromboxane A_2 (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 2994). This highly unstable compound (half-life in water about 30 s) is formed from the same precursor as the prostaglandins (arachidonic acid through an unstable endoperoxide, prostaglandin G_2), but differs sufficiently in structure from the prostaglandins to justify the use of new nomenclature. Thromboxane A_2 (which is almost certainly identical to the rabbit aorta-contracting substance previously discovered by Piper and Vane) is a powerful stimulant of platelet aggregation. It is probable that the formation of this substance is responsible for the clumping of platelets which, if allowed to continue inside a blood vessel, may result in thrombus formation. If the blood vessel becomes completely occluded by such a thrombus, the consequent tissue damage, for example in the heart or brain, may prove rapidly fatal.

Oates and his colleagues at Vanderbilt University have reported (*Science*, **193**, 1135; 1976) that thromboxane A_2 released from human platelets can constrict coronary blood vessels of the pig. They postulate that platelet aggregation in areas of damaged endothelium can release thromboxane A_2 and thus cause constriction of large coronary arteries.

A more recent development is reported in this issue of *Nature* (page 663). It comes from Vane's group at the Wellcome Research Laboratories, Beckenham. In the microsomal fraction of homogenised blood vessels they have discovered an enzyme which converts the endoperoxide intermediary, prostaglandin G_2, not to the classical prostaglandins (E_2, $F_{2\alpha}$ and D_2) nor to thromboxane A_2 (as occurs in platelets) but to a new substance (PGX) which has not yet been identified. PGX has two important pharmacological properties both opposite to those of thromboxane A_2. It is the most powerful inhibitor of platelet aggregation yet discovered and it dilates blood vessels. Thus the endothelial lining of blood vessels possesses its own mechanism for generating a substance which will prevent platelets from adhering to its surface or to each other. Moreover, the substance will tend to maintain the flow of blood by dilating the vessel itself.

The following sequence of events can therefore be envisaged. When platelets come into contact with endothelium of the normal blood vessel wall they release a quantity of the prostaglandin endoperoxide, which is then used as a substrate by the blood vessel enzyme to form PGX. By its inhibitory properties this substance will, in turn, counteract any tendency of platelets to aggregate, or of the blood vessels to constrict. If, however, platelets come into contact with blood vessels whose endothelium has been disrupted or destroyed by pathological changes, there will be no enzyme for the formation of PGX and so the aggregating action of thromboxane A_2 formed by the platelets from prostaglandin endoperoxide, will go unopposed leading to thrombosis and local vasoconstriction.

Since PGX, like thromboxane A_2, is an unstable compound, its usefulness as a drug to treat or prevent thrombosis in man may be limited. Nonetheless, the elucidation of its structure is of paramount importance. Once the structure is known, analogues can be synthesised with the object of finding a compound which combines the pharmacological (PGX-like) activity with greater stability. Such a drug could be used in both the treatment and prevention of thrombosis.

Thrombosis is one of the most common causes of death in the western world. Young and middle-aged men, apparently fit and in the prime of life, are frequent victims. Few really effective remedies or preventative measures are available. Until now, in spite of much outstanding research, the sequence of events which leads to thrombosis at what is popularly called the 'molecular level' has been incompletely understood. The potential value to medicine of the discovery of PGX is therefore incalculable.

the relative importance of the climatic controls, which is an essential step towards climatic prediction, collaboration between people using often widely divergent methods to study climatic change is highly desirable.

The recent article by Colebrook (*Nature*, **263**, 576; 1976) highlights one area where such collaboration should prove fruitful. Colebrook analyses long series of oceanographic and climatic data in order to define changes in the North Atlantic current systems and ocean temperature during the past 100 yr and to suggest ways in which shifts in the ocean and atmospheric circulation are related. He concludes that, on time scales of decades, sea surface temperature changes are largely due to variations in advection associated with the changing intensity of the Gulf Stream and North Atlantic Drift currents. He states that these currents are responding to variations in the atmospheric circulation over the North Atlantic, although the atmospheric circulation index chosen (tropical cyclone frequency) is remarkably indirect.

Colebrook also shows, contrary to other widely publicised opinions, that recent changes in the oceanic climate are not unusual in the context of the past 100 yr. In addition, he presents further evidence of the approximately 10-yr periodicity in sea surface temperature in the North-East Atlantic that has been mentioned by other investigators. He links this periodicity to changes in atmosphere pressure associated with the sunspot cycle, as given by Parker (*Met. Mag.*, **105**, 33; 1976). It is interesting that the connection between variations in the strength of the East Atlantic meridional atmospheric flow and solar activity is also apparent in the 24-h change in atmospheric pressure distribution following a solar flare (see, for example, Schuurmans, *Meded Verh. K. ned. met. Inst.*, **92**; 1969). The effects on climate of solar variation are gradually being accepted as genuine but of secondary importance, tending to modify the nature of the meridional rather than the dominant zonal atmospheric air flow.

Increasing evidence of frequency-dependent relationships between climatic variables is coming to light. Sea surface temperature to the west of the United Kingdom seems to be controlled by the strength of the broad Atlantic westerly air flow on time scales of decades and longer. Yet, on the shorter time scale of the 10-yr periodicity, local changes in the meridional component of the atmospheric circulation are more important. This association between sea temperature and southerly air flow in the East Atlantic on short time scales has been demonstrated by Dickson (*Dt. hydrogr. Z.*, **24** (3), 97; 1971).

Investigations such as this are hampered by the lack of long climatic data series. As usual when limited data are

available, hypotheses multiply, each able to explain the observations. The contribution of such empirical analyses to the search for the causes of climatic changes can be maximised if they are used in conjunction with the theoretical approach to the same problem: numerical modelling. The numerical modelling of climatic change and the general circulation of the atmosphere is at an early stage of development; such experiments are primarily designed to test the behaviour of the models, using unrealistically large anomalies in the boundary conditions to produce an unequivocal response in the atmosphere. Until reliable coupled ocean–atmosphere models become available, numerical models can only provide a static picture of a climatic state and cannot portray climatic evolution. Their potential, however, is great and slowly being realised. Hypotheses concerning the causes of climatic change based on empirical evidence can be tested by numerical simulation and *vice versa*.

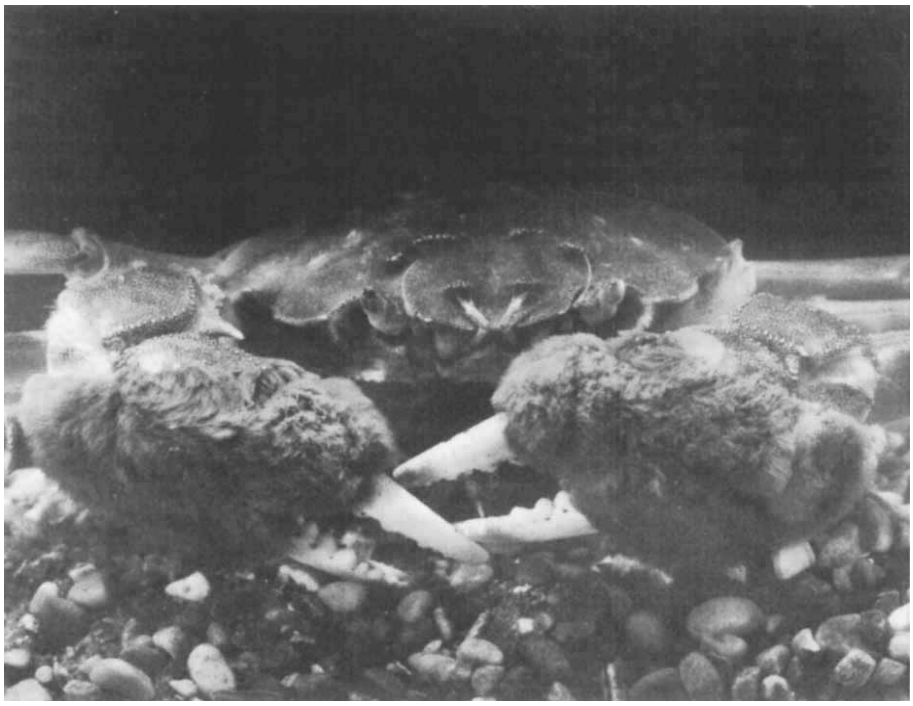
To assess the relative importance of the climatic controls, it is necessary to specify realistic anomalies in the boundary conditions of the models. Otherwise the value of the experiment is lessened. It may indicate that a particular control can be effective, but not how effective. The use of information concerning actual changes in the boundary conditions of the atmosphere, such as contained in the article by Colebrook, should ensure that the results of numerical modelling experiments do not diverge unnecessarily from reality.

Increased collaboration between people studying climatic change within a theoretical framework and those concentrating on the observational data is highly desirable, and perhaps essential if climatology is to provide some indication of future climatic development. □

Chinese mitten crab reappears in Britain

from R. W. Ingle and M. J. Andrews

THE unintentional transportation of animals by man beyond the range of their normal habitats has always interested zoogeographers. Five species of true crabs have arrived in British waters in this manner. One of these, the Chinese mitten crab, is of particular interest as its spread through Europe since its introduction from China has been well documented and because it has now reappeared in Britain after an apparent absence of some 27 yr.



Front view of the male mitten crab from West Thurrock generating station. This species is easily recognised by the dense fur on the claws. The body colour varies from greenish grey to dark brown.

The mitten crab, *Eriocheir sinensis* A. Milne Edwards, is a native of temperate eastern Asia and occurs from the Fukien province of China to the western coastal region of Korea. In Korea it shows preference for rice fields near the coast and inland occurs only in rivers. Life in this temperate environment has enabled the crab to adapt to similar climatic conditions in northern Europe from where it was first reported in 1912 from the Weser River near Retham, Germany and later from other parts of the country. In the 1920s the crab spread rapidly westward and was recorded from rivers in the Netherlands, Belgium, France, Denmark and Finland. Its occurrence in Britain was first reported in 1935 when a male was found in the River Thames at Chelsea, then in 1949 a second specimen was discovered in Southfields reservoir near Castleford, Yorkshire. There have been no other reports of this crab in Britain until this year when three specimens were taken from the cooling water intake screens of West Thurrock electricity generating station in February, May and June. The West Thurrock station is situated in a brackish region of the River Thames estuary 36 km below London Bridge where the average half-tide salinity for the first half of 1976 was approximately 20 g per 1,000 g.

The mitten crab probably reached Europe in the ballasting tanks of commercial vessels, having entered the tanks in Chinese waters as free swimming larvae and developed *en route* to be liberated as juveniles when the tanks were emptied in Germany. Studies in Europe have shown that adult crabs prefer freshwater and live in rivers but migrate into estuaries to breed. The eggs are hatched in brackish water and developing larvae move gradually back into freshwater.

This recent reappearance of the mitten crab in the Thames is interesting because all the specimens were found within a period of 5 months and in an area that has been surveyed regularly since the 1960s, during which time the crab was never reported. It is probable that these individual crabs were transported to the West Thurrock region by ships arriving from European ports (more than 100 ships from Antwerp and Rotterdam have visited West Thurrock in the past 2 yr), and does not constitute a serious invasion by the species. In large numbers mitten crabs can cause considerable damage to estuarine river banks resulting from the subsidence of their numerous small burrows in the mud. The crab is also reported to cause damage to fishing nets by cutting the mesh in which they often become entangled. There seems no obvious reason why *Eriocheir* should not become established in the British Isles and the arrival of females carrying eggs would be a significant step towards this invasion; further occurrences of the mitten crab are awaited with interest. □

Rheology at Gothenburg

from D. R. Oliver

The Seventh International Congress in Rheology was held at Chalmers University of Technology, Gothenburg, Sweden on August 23–27, 1976, organised by Professor Josef Kobat and colleagues (Chalmers University).

RHEOLOGY is the study of the deformation and flow of materials, but a visit to Gothenburg provided opportunities to observe the deformation and flow of travellers cheques! The subject subdivisions chosen by the organisers were polymer solids, polymer melts, fluids, polymer solutions, theory, suspensions, biorheology, methods, metals, concrete and food. This order represents a ranking in terms of numbers of papers presented: new subjects such as biorheology (13 papers) and food (4) seem to be making little impact on the combined polymer topics (149). The apparent fall in the number of theoretical papers (23) is illusory, since papers under other headings contain extensive theoretical work and one new concept in particular seems to be gaining momentum, the search for rheological equations of state consistent with thermodynamics (J. G. Oldroyd (University of Liverpool) and G. Astarita (University of Naples)). Another link between rheology and physics was provided in a historical lecture by H. Markovitz (Carnegie–Mellon University, Pittsburgh) who pointed out that 100 yr ago Boltzmann formulated his principle of superposition, according to which stresses present at a given time depend not only on the deformation at the time but also on previous deformations, the influence of which decreases with increasing elapsed time. The suggestion was made that Boltzmann's ideas should be given status comparable with those of Maxwell in the early development of theories of viscoelastic behaviour.

Flow visualisation is widely used in rheology: some unusual and beautiful extrudate flow forms were shown by N. Bergam (CIIR, Oslo) and die-entry flows, with customary flair, by Professor Giesekus (University of Dortmund). The quadruple vortex formed in a Taylor 4-roll mill was demonstrated by J. M. Broadbent (University of Wales, Aberystwyth) and the flow pattern around a sphere moving in a viscoelastic liquid by D. Sigli and M. Coutanceau (University of Poitiers). The pattern of a recoiling flow upstream of an orifice upon sudden cessation of flow was shown by R. J. Gordon (University of Florida) and a remarkable tri-modal Weissenberg effect induced by torsional oscillation of a rod in a viscoelastic liquid by D. D. Joseph (University of Minnesota). The liquid climbing the rod builds up into three (or even four) distinct lobes before eventually collapsing.

A computational method finding increasing acceptance is the finite element system, in which the uniform grid of a finite difference network is replaced by a set of irregular elements which concentrate attention around areas of particular interest. R. Tanner (University of Sydney) obtained valuable information regarding velocity profiles at the exit end of a capillary tube and was able to predict jet swell for Newtonian fluids; N. Davids (Pennsylvania State University) and M. L. Wenner (General Motors, Michigan) used finite elements for the study of uniaxial waves in a viscoelastic rod; D. S. Malkus (National Bureau of Standards, Washington) calculated hole pressure error, and G. Geymonat (CNRS, Marseilles) and M. Raous (Poletecnico, Turin) applied the technique to the near-solid problems of the behaviour of turbine blades and to time-dependent effects in ageing concrete. The finite element method will become widely used in rheology and other branches of fluid flow.

The flow of blood was the dominant theme of the biorheology section. New viscosity concentration relationships were obtained by D. Quemada (University of Paris) and hysteresis effects in whole human blood in Couette flow studied by A. Apelblat *et al.* (Paris). These experiments showed that the aggregation of red cells is both a time- and shear-dependent phenomenon producing hysteresis loops in rotary instruments. The possible migration of red cells away from the walls should not be ignored; these effects occur strongly in flow down fine capillaries and produce the Fahraeus–Lindquist effect, that is, lower than normal apparent blood viscosity. M. Singh (IST, Madras) and N. A. Coulter (University of North Carolina) studied the latter behaviour in oscillatory flow down capillaries and reached the interesting conclusion that the effect begins at larger tube radii than for steady flow—this may help the human body considerably! In a significant paper L. E. Gelin (University of Gothenburg) showed how the rheological changes in blood following shock or trauma influence its distribution in fine capillaries. A Perspex model allowed blood to flow along capillaries and then to choose a "straight on" or "branching" path. For highly aggregated blood (such as that following shock), the forward flow contained more red cells and the side flow less, particularly for very narrow tubes. In critically ill patients, this may lead to the total immobilisation of red cells, visible as "red shock".

Several new concepts were introduced. B. Mena and O. Manero (National University of Mexico) showed that the flow rate of a viscoelastic liquid along a tube could be increased 10-fold by the low frequency axial oscillation of the tube, producing spectacular spurts of liquid. A paper by K. Walters (University of Wales, Aberystwyth) described his "torsional balance" variant of the rheogoniometer, which started life as a squeeze film system but has become a potential rival to jet thrust as a high-shear steady-state normal stress measuring instrument.

Extensional measurements continue. L. Nicolais (University of Naples) and colleagues showed that the extensional viscosity of polymer relations containing fine glass beads in suspension is actually reduced due to the presence of the filler; conversely D. R. Oliver and R. C. Ashton (University of Birmingham) showed that the polymers added to car lubricating oils cause large increases of extensional viscosity in both uniaxial and biaxial flows.

A novel fibre foam was described by S. Turner and F. N. Cogswell (ICI, Welwyn Garden City). This fibre/resin/air matrix is easily bonded to other materials and is light and strong. The uses suggested include shock absorbent packing, fenders and riot shields—certainly a topical development. □

A hundred years ago

THE Fellows of the College of Physicians of Dublin have deliberately determined to admit Miss Edith Pechey to the examination for the L.K.Q.C.P.I., and have thus thrown open the portals of the medical profession to all comers, whether they be "persons" of the male or female sex. However pregnant of results this decision may be, says the *Medical Press and Circular*, it does not seem to us that any other conclusion was possible, and we expect to see a similar ingress allowed to the ladies by all other bodies. The Queen's University, it is anticipated, will be the next to follow suit, and these fortresses having surrendered at discretion, it is impossible that others can long sustain the seige.

From *Nature*, **14**, October 19, 560; 1876.

Interferon therapy for hepatitis

from Arie J. Zuckerman

THE major importance of persistent infection with hepatitis B virus and the carrier state can be considered first in terms of the potential dissemination of the infection to contacts by various parenteral (direct introduction into the tissues) and non-parenteral routes; and there are, at a conservative estimate, some 110 million carriers of hepatitis B surface antigen. Second, a significant proportion of infected patients and carriers have chronic active hepatitis, cirrhosis and probably primary liver cancer in certain areas of the world. Much has been written on this subject and considerable effort is being directed at passive and active immunisation. There is, however, no really effective treatment which affects the course of chronic infection with hepatitis B virus, but somewhat unexpected and encouraging results have now been reported after systemic treatment with exogenous interferon, first by Greenberg and associates (*New Engl. J. Med.*, **295**, 517; 1976) and more recently by Desmyter and colleagues (*Lancet*, **ii**, 645; 1976).

Interferon has a well known broad spectrum antiviral action, and its lack of antigenicity in the homologous species and the absence of toxicity immediately provide a compelling appeal for its use. Indeed interferon has been investigated intensively since its discovery in 1957 by Isaacs and Lindenmann, and much effort has been devoted to its application in clinical practice for the treatment of several viral infections (Merigan *et al.* in *Antiviral Mechanisms, Perspectives in Virology*, **9** (edit. by Pollard, M.), 249; Academic, 1975). Interferon is probably not a single substance but rather it represents a class of cellular glycoproteins occurring as monomers or polymers of a basic unit with a molecular weight of 12,000–20,000. It is synthesised by all vertebrates studied in response to viral infection and other intracellular parasites, polyanions, endotoxin and certain low molecular bis basic organic compounds. It is also produced as part of the cell-mediated immune response by lymphocytes.

There is experimental evidence that the viral inhibitory effect of interferon is at the level of translation of viral proteins; other data indicate that transcription of the viral genome is inhibited. But it now seems from other evidence that the viral inhibitory effect may be exerted not by interferon itself but by a cellular protein induced by interferon, and further that there is a range of proteins that can be considered as "interferons". Interferon also has an inhibitory effect on rapidly dividing cells including tumour cells, and its antitumour potential is under investigation. Interferon can be expected, therefore, to be more effective for prophylaxis than for the treatment of established virus diseases. However, in terms of disease process resulting from continuing viral replication and spread, interferon may be useful, for example by preventing the infection of fresh cells.

Greenberg *et al.* investigated the effect of exogenous human leukocyte interferon on persistent hepatitis B virus infection associated with chronic active hepatitis in four patients. The interferon was produced by Dr Kari Cantell by stimulation of human blood buffy coats with Sendai virus. The specific activity of the interferon was 5×10^5 units per mg protein. The four patients selected for the investigation were carriers of hepatitis B surface antigen for more than 6 months, and they had persistently abnormal liver function tests and histological changes in the liver consistent with chronic active hepatitis. In addition, three of the patients had high levels of circulating Dane particle markers including DNA polymerase, core antigen and Dane particle-associated DNA. Hepatitis B *e* antigen was present in these three patients, and the fourth patient had *e* antibody (see *Nature*, **263**, 374; 1976). The administration of interferon in a dose of between 6×10^3 and 17×10^4 units per kg per d was associated with a rapid and reproducible fall in DNA polymerase activity and the other Dane particle markers in the three patients on five separate occasions. This effect was transient when the interferon was given for up to 10 d, but seemed to be more durable when the course of treatment lasted for a month or longer. Prolonged therapy was associated with the disappearance or diminution of the *e* antigen in two of the patients. The effect of interferon on the surface antigen was more variable. Short term treatment had no effect on the titre of the surface antigen, but treatment for a month or longer was associated with a significant fall in titre in two patients.

Interferon thus seems to be exerting a suppressive effect on the production of Dane particles, the presumed infectious agent of hepatitis B. It is not known, however, at what stage of assembly of the Dane particle this effect occurs. Furthermore, interferon has also been shown to affect both humoral and cellular immunity, and Greenberg and associates point out that the suppression of the markers associated with hepatitis B virus may have been due to a direct antiviral action or mediated by the immune system or a combination of both. There is also the possibility that the active principle is not interferon but some other lymphokine including transfer factor (*Nature*, **258**, 14; 1975) or other cell product which is present in the partially purified preparation collected from the human blood leukocytes. But this seems unlikely in view of the essentially similar results obtained by Desmyter *et al.* (*loc. cit.*) with interferon prepared in cultures of human diploid fibroblasts using the double-stranded RNA, poly(I) · poly(C), and superinduction. This preparation was given to two chimpanzee persistent carriers of hepatitis B surface antigen and one patient with antigen-positive chronic aggressive hepatitis. The treatment consisted of seven doses of 10^7 international units of human interferon given on alternate days for 2 weeks. Interferon had no effect on the titre of the surface antigen of the two chimpanzee carriers but striking changes were observed in the hepatitis B core system. In the first chimpanzee, 15% of the nuclei of liver cells were found positive for core antigen by immunofluorescence. After treatment for 1 week, the number of positive nuclei and the intensity of staining decreased progressively during 5 weeks, and at that time 0.5% of the nuclei were positive. Then the number of positive nuclei increased rapidly reaching 30% after 1 month. The titre of circulating core antibody also increased. The second chimpanzee had no demonstrable core antigen in the liver cells at any time, but after treatment the high titre of serum core antibody fell sharply. In the patient, changes were seen in the core system. Intense core antigen staining was found in 30% of the liver cells before treatment. Immediately after treatment, 20% of the liver cell nuclei were positive at low intensity, and 5 weeks later only 3% were positive. The titre of core antibody in the serum remained unchanged.

These preliminary results obtained in short-term investigations in a few patients are promising and potentially most important, since interferon therapy may be useful in limiting the infectivity of carriers and it may perhaps eradicate chronic infection. The experimental hepatitis B vaccines currently under development are unlikely to achieve this in the foreseeable future. However, even the most ardent proponents of interferon and lymphokine therapy will urge carefully designed studies and only cautious optimism, for much remains to be learnt and accomplished before this form of treatment can be applied successfully to the many important clinical problems so clearly outlined by Merigan and his colleagues. □

review article

RNA polymerase specificity and the control of growth

Andrew Travers*

RNA polymerase is an allosteric enzyme whose activity and specificity are controlled by interaction with a nucleotide, tRNA and proteins. In E. coli *the availability of these effectors should ensure that the quality of transcription is tightly coupled both to translation and to the metabolic state of the cell.*

RNA polymerase is structurally one of the most complex enzymes in the bacterial cell. This key component of the machinery for the expression of genetic information is a heteromultimer of molecular weight $\sim 5\times10^5$ (ref. 1). Yet, in the case of the mitochondrial RNA polymerase a single polypeptide chain of molecular weight $\sim 6\times10^4$ suffices for the actual process of transcription[2]. The complexity of the bacterial enzyme must thus reflect additional roles in the regulation of transcription and DNA replication. One aspect of this control is promoter selection. In this review I argue that RNA polymerase is an allosteric enzyme with a variable initiation specificity and can discriminate between different types of promoter site. The molecular basis for such discrimination is the ability of the polymerase to exist in functionally distinct forms, each of which preferentially initiates at a particular class of promoter sites. One effector, the nucleotide ppGpp, whose intracellular level is tightly coupled to both protein synthesis and the metabolic state of cell[3], alters the specificity of the polymerase by converting one form of the enzyme to another. Other effectors include fmet $tRNA_f^{met}$ and EF-TuTs, both of which are intimately associated with translation. The emergent pattern of transcriptional control is thus one in which RNA polymerase itself has a central role in the determination of the quantity of the transcript. The purpose of this control is to ensure that the production of ribosomes and mRNA is balanced and geared to available energy and to the rate of protein synthesis. On this model the mutual interaction of polymerase and specificity modulators thus constitute a mechanism for the control of growth.

RNA polymerase specificity

The extent to which the direct control of RNA polymerase activity complements the operation of highly specific transcriptional controls by DNA-binding repressors and activators during normal bacterial growth has remained poorly understood. To establish a regulatory role for the transcribing enzyme in the selection of genetic information it is necessary to demonstrate both that the purified polymerase can be directly controlled *in vitro* and that mutations resulting an an alteration in polymerase structure significantly alter the pattern of transcriptional regulation *in vivo*.

One possible mode of regulating RNA polymerase activity would be the control of promoter selection. The binding of RNA polymerase to a promoter site is the first step in the synthesis of an RNA molecule and a major determinant of the initiation frequency *in vitro*. Following this initial binding there is a change in the conformational state of the DNA in the promoter region[4,5]. Once this transition has occurred the polymerase can initiate an RNA chain very rapidly[6]. The transition between the DNA conformational states is reversible and often occurs over a narrow temperature range, the midpoint of which defines a transition temperature, t. With a given set of conditions *in vitro* each promoter will have a characteristic transition temperature for opening by RNA polymerase holoenzyme. The transition temperature is dependent on factors which directly affect the structure of the DNA or which alter the affinity of the enzyme for the promoter site. Thus a change in initiation specificity of RNA polymerase can be defined as a differential alteration in t between promoters.

Experimentally two criteria can be used to measure the relative affinity of RNA polymerase for different promoters. In this context the term affinity describes the strength of a polymerase–promoter interaction which is necessary for the formation of stable complexes without any qualification as to the exact molecular requirements for this interaction, that is as to whether or not binding to the DNA double helix in the promoter region is sufficient. One criterion is the measurement of the temperature dependence of polymerase–promoter complex formation, a decrease in the affinity of the enzyme for the promoter being reflected in an increase in t. A second criterion is the measurement of the response of a particular type of RNA synthesis to ionic strength. Increasing salt concentration reduces the binding of polymerase to the template concomitantly increasing t[7]. Thus at a constant temperature a decreased affinity of RNA polymerase for a particular promoter may be reflected in an increased salt sensitivity of transcription of the cognate RNA. Neither of these criteria is ideal since the physical structure of RNA polymerase is also to some extent dependent on both ionic strength[8] and temperature[9] and it cannot be excluded that these structural changes might in themselves alter the pattern of transcription. Nevertheless used together these criteria provide useful indicators of the relative affinities of polymerase for different promoters.

ppGpp—an effector of RNA polymerase specificity

In vivo any alteration in the initiation specificity of RNA polymerase might be expected to yield a wide ranging change in the pattern of RNA species synthesised. One such

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change occurs on starvation of certain bacterial strains for a required amino acid. In this stringent response the accumulation of stable RNA species, both rRNA and tRNA, is severely restricted while, in contrast, the production of certain mRNA species, for example, φ80 mRNA[10], is relatively unaffected. The reduction in rRNA accumulation is primarily a consequence of the diminished initiation of rRNA chains. Another rapid response to amino acid starvation is the intracellular accumulation of the guanosine nucleotide, ppGpp, to millimolar levels within seconds of the onset of starvation[11]. Mutants unable to accumulate ppGpp in this manner fail to shut off rRNA synthesis and are said to show relaxed control[12]. This correlation has led to the proposal that this nucleotide is the mediator of the stringent response *in vivo*[12].

There is considerable evidence that *in vitro* ppGpp can preferentially inhibit the synthesis of rRNA relative to total transcription both in crude[13,14] and in highly purified systems[15-18] At issue is the manner in which nucleotide effects this inhibition; whether RNA polymerase itself is the target or whether additional transcription factors are required. Early studies showed that ppGpp inhibited total RNA synthesis by purified *Escherichia coli* RNA polymerase on a variety of DNA templates[15,19]. The specificity of this inhibition can be determined by comparing the effect of ppGpp on the synthesis *in vitro* of rRNA with the synthesis of φ80 RNA, an RNA species which is not stringently controlled *in vivo*, using as template the DNA of a transducing phage, φ80 d_3 rrnB$^+$ ilv$^+$ su$^+$7, containing both φ80 and rRNA promoters. Such a comparison shows that ppGpp increases *t* for opening the rRNA promoter by 10–15 °C; without the nucleotide *t* at 0.075 M KCl is 25–30 °C, in the presence of ppGpp *t* is 35–40 °C (ref. 17). In contrast ppGpp has little effect on *t* for opening φ80 promoters (for example, Fig. 1). ppGpp acts by inhibiting the formation of polymerase–rRNA promoter complexes and is without significant effect on the subsequent extent of RNA synthesis once such complexes have formed. Thus ppGpp clearly alters the initiation specificity of RNA polymerase holoenzyme *in vitro* in a direction which parallels the stringent response *in vivo*.

How does ppGpp alter the pattern of initiation by RNA polymerase? The experimental evidence shows that the effector alters the properties of RNA polymerase itself. To demonstrate this the enzyme is first incubated with ppGpp in the absence of nucleoside triphosphates or templates for a given time. Then the polymerase is assayed by addition to a reaction mixture containing these components but no additional ppGpp. When the enzyme is tested immediately after mixing with ppGpp an initial inhibition of all types of RNA synthesis is observed[17]. Thereafter on further preincubation the specificity of transcription slowly changes so that the enzyme recovers almost entirely the ability to transcribe φ80 and T2 DNA but its capacity to synthesise rRNA diminishes further, plateauing at ~20% of the control value. During this change no detectable degradation of ppGpp is observed. The half time of this switch in specificity is 30–50 s. Functional removal of ppGpp by dilution reverses the change in specificity with approximately the same kinetics. Other guanosine nucleotides, for example ppG, do not produce this effect. Thus the specificity change induced by ppGpp is reversible and is specific for the nucleotide. Although the rate of change of transcription specificity is slow by comparison with other protein isomerisation constants, the immediate inhibition of polymerase activity on mixing enzyme and nucleotide suggests that this is not a consequence of a slow simple association of the two components. Rather the data are compatible with a fast association followed by a slow structural change in the enzyme. The nature of this change is discussed below.

Molecular basis of RNA polymerase specificity

The differential response of the synthesis of RNA species to ppGpp establishes that RNA polymerase by itself can discriminate between different types of promoter and that this discrimination can be regulated *in vitro*. The promoters themselves must thus differ in recognition parameters for the enzyme.

How is this discrimination effected at the molecular level? The properties of RNA polymerase isolated from *E. coli* infected with phage T4 provide a clue. After T4 infection a selective shut off of stable RNA synthesis occurs, a process which requires an unidentified T4 gene product[20-22]. In addition RNA polymerase is structurally altered. One change, termed modification[23], involves the covalent addition of an adenosine diphosphoribose residue to each α subunit[24] while in addition three small phage-induced polypeptides are found associated with the core RNA polymerase[25-27]. When core polymerase from T4-infected cells is supplemented with σ factor from uninfected cells, a requirement for rRNA synthesis[28], the reconstituted holoenzyme has a lower affinity for rRNA promoters than holoenzyme from uninfected cells[29]. Indeed the qualitative pattern of rRNA synthesis by "T4 holoenzyme" is very similar to that of normal holoenzyme in the presence of ppGpp. The transition temperature for opening the rRNA promoter is high, ~35–40 °C, and rRNA synthesis is very salt sensitive. Further the ability of polymerase to transcribe phage DNA templates is relatively unimpaired after T4 infection. The reduction in affinity of the enzyme for rRNA promoters is

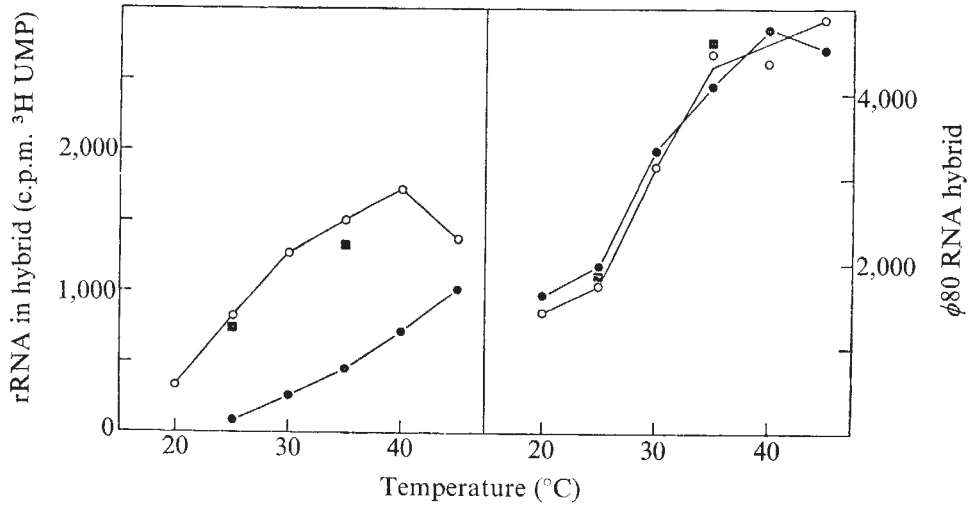


Fig. 1 Effect of temperature and 0.2 mM ppGpp on φ80 RNA and rRNA synthesised from preformed promoter–polymerase complexes on φ80 d_3 DNA. Promoter polymerase complexes were formed by incubation of template and enzyme at the indicated temperature for 15′ and were assayed by the simultaneous addition of heparin and the nucleoside triphosphates: ○, control; ●, preincubation and assay in presence of 0.2 mM ppGpp; ■, 0.2 mM ppGpp added together with heparin and nucleoside triphosphates. Data are taken from ref. 17 where a full description of methods can be found.

also shown by polymerase prepared from cells infected with a T4 mutant unable to modify the α subunits[30] and is probably a function of a small phage coded polypeptide of molecular weight 15,000.

Thus at least two types of rRNA synthesis can be distinguished by purified RNA polymerases. With *E. coli* holoenzyme alone t for opening the rRNA promoter is 25–30 °C; that for *E. coli* holoenzyme in the presence of ppGpp and for T4 holoenzyme is 10–15 °C higher. Thus RNA polymerase can exhibit either a low or a high affinity for the rRNA promoter when that promoter is on the DNA of a transducing phage. However, when *E. coli* DNA is a template *in vitro* in conditions of limiting polymerase, rRNA synthesis by *E. coli* holoenzyme has the characteristics of rRNA synthesis by T4 holoenzyme[28,31]. The transition temperature is high and is not increased by ppGpp. This shows that *E. coli* holoenzyme can also exhibit a low affinity for rRNA promoters in the absence of ppGpp and suggests that the enzyme may exist as an isomeric mixture of two, or more, forms which differ in their affinity for rRNA promoters, and that the form for rRNA synthesis is 'used up' on *E. coli* DNA.

Theoretically RNA polymerase could exist either as a single form with a uniquely defined specificity or in a small number of conformational states, each of which would have a preferred affinity for a particular class of promoter sites. On the first model discrimination would depend solely on the relative affinity of the enzyme for particular promoters while on the second discrimination would also depend on the distribution of the enzyme between the different states.

These formal possibilities can be distinguished experimentally by template competition experiments *in vitro*. First, a system is characterised in which RNA synthesis proceeds simultaneously from two types of promoter, for example, phage and ribosomal RNA promoters. This system is then perturbed by the addition of increasing quantities of another DNA template. This DNA will cause a redistribution of polymerase molecules by competing for the enzyme. If polymerase exists as a single form with invariant specificity, the relative competition of RNA synthesised from different promotors should be independent of the competing template and depend only on the relative affinity of the enzyme for the respective promoters. In contrast, if the enzyme exists in multiple forms each with differing initiation specificity the character of the competition observed should depend on the nature of the competing template. In a mixed template system containing $\varphi 80\, d_3$ rrnB$^+$ ilv$^+$ su$^+$7 DNA and T2 DNA, addition of T7 DNA results in a reduction of T7 RNA synthesis and a stimulation of rRNA synthesis[32]. By contrast, addition of *E. coli* DNA reduces the synthesis of both rRNA and T2 RNA but completes rRNA synthesis to a greater degree. At the same time the residual rRNA synthesised in the presence of *E. coli* is no longer preferentially sensitive to ppGpp. Thus the character of the competition observed depends on the nature of the competing template, suggesting that RNA polymerase molecules do form a mixed population in which the enzyme can assume two, or more, recognition states. By contrast, in the presence of ppGpp the relative competition of T2 RNA and rRNA synthesis is virtually dependent of the nature of the competing template. These results also imply that *E. coli* DNA preferentially binds a form of the enzyme which has a high affinity for rRNA promoters and is selectively inhibited by ppGpp. Consequently rRNA synthesis from *E. coli* DNA by this form of the enzyme can only be detected after titration of the competing binding sites.

These arguments support the hypothesis that RNA polymerase holoenzyme exists in functionally distinct forms each of which preferentially initiates at a particular class of promoter sites (Fig. 2). Holoenzyme may exist in at least two forms, one with a high affinity for rRNA promoters, the second with a low affinity. In the presence of ppGpp and after T4 infection the enzyme exhibits the activity of principally the low-affinity rRNA form, while retaining the ability to transcribe phage DNA efficiently. Thus the low-affinity rRNA form may have a relatively high affinity for phage promoters.

What is the mechanism of the alteration of initiation specificity by ppGpp? The nucleotide could act either by directly inactivating one class of polymerase molecules, thereby reducing the total amount of enzyme available, or by converting the high-affinity rRNA form into the low-affinity rRNA form. The kinetics of the change in specificity induced by ppGpp are compatible with conversion. Further, low concentrations of ppGpp increase transcription of phage DNA templates in conditions of DNA excess suggesting that the nucleotide can increase the amount of enzyme able to transcribe phage DNA efficiently. This type of evidence suggests that the specificity isomers of RNA polymerase are interconvertible.

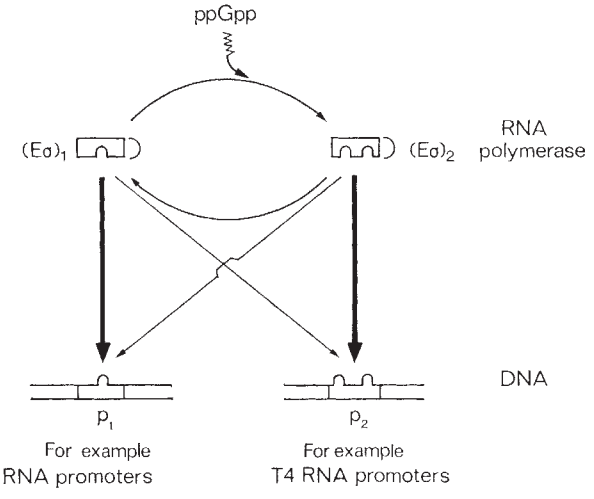
The crucial question is whether the variations of initiation specificity observed *in vitro* are biologically relevant. In two instances where stable RNA synthesis is shut off *in vitro*, ppGpp accumulation and T4 infection, the affinity of RNA polymerase for rRNA promoters *in vitro* is reduced. In the latter case there is good evidence that this parallel reflects the situation *in vivo*. Recently mutants of T4 have been isolated which fail to shut off host RNA synthesis normally. RNA polymerase prepared from cells infected with these mutant phages lacks the 15,000 dalton T4-coded polypeptide (Gold, Kutter, Sirotnik and Snyder; Snustad, personal communications) which the *in vitro* studies had implied was necessary for the reduction of the affinity of polymerase for rRNA promoters.

Other types of mutant RNA polymerase should also exist. One would be insensitive to ppGpp and phenotypically relaxed. A second type would have an altered distribution of different forms of the enzyme with a consequent modified response to ppGpp. *In vivo* such a mutant would have an unbalanced pattern of gene expression. No mutants of the first class have yet been isolated but one of the second class has been characterised.

This mutant, *ts*103[33], is unable to support the growth of lambdoid phages and has an altered pattern of protein synthesis. The isolated polymerase behaves as though the equilibrium between the high- and low-affinity rRNA forms is shifted in favour of the high-affinity isomer. The mutation maps at a locus distinct from those defining known subunits of RNA polymerase (Baralle *et al.*, manuscript in preparation).

An important consequence of promoter recognition by

Fig. 2 Multiple form model for RNA polymerase specificity. This is the simplest model assuming only two forms.



multiple forms of RNA polymerase is the possibility that the relative affinity of different forms of the enzyme for a promoter can vary. Thus at one extreme a promoter may bind tightly only one polymerase isomer, while in contrast another promoter may interact equally well with two or more forms of the enzyme. In this way a virtually continuous spectrum of recognition characteristics can be generated so that the promoter for any particular transcriptional unit is adapted to the control of polymerase specificity in a manner appropriate to the cell's requirements. Such a model predicts that the responses of the synthesis of particular RNA species to ppGpp will not fall into a small number of distinct classes but rather that the synthesis of each RNA species will have a characteristic individual response. The available evidence *in vitro* supports this view. Thus in a crude cell lysate the responses of the synthesis of four proteins, β-galactosidase, EF-Tu, β and β' subunits of RNA polymerase and ribosomal protein L7/L12 to ppGpp are quantitatively very different[34].

For simplicity I have so far considered RNA polymerase specificity formally in terms of two isomers. One indication that the regulation of promoter recognition by the enzyme may be more complex is the observation that in an *in vitro* system containing purified RNA polymerase the K_i for the preferential inhibition of su_3^+ tRNA synthesis from φ80 psu_3^+ DNA and of poly(G) synthesis from poly d(I-C) is 5 μM (refs 35 and 36). This contrasts with the value of 150 μM for rRNA synthesis and suggests that RNA polymerase may exist in at least three forms. Significantly, concentrations of ppGpp up to 10 μM which strongly inhibit su_3^+ tRNA synthesis substantially increase rRNA synthesis from φ80 d_3 DNA (Fig. 3).

Coupling of transcription and translation

I have argued that ppGpp alters polymerase specificity by converting one form of the enzyme to another. The rapid synthesis of this effector by an idling reaction on the ribosomes[12] is triggered by the codon-dependent binding of uncharged tRNA to the acceptor site[37,38]. The steady state concentration of the nucleotide is thus coupled to and requires a functioning ribosome. In addition, the rate of degradation of ppGpp is dependent on the energy input of the cell such that breakdown is facilitated when available energy is high[39,40]. In this way the level of ppGpp in the cell, and hence its effect on transcriptional specificity, is geared to both protein synthesis and energy metabolism.

Evidence *in vivo* shows that ppGpp, although a major effector of transcriptional specificity, may not be the sole controlling element. The close involvement of transcription with translation is suggested by the suppression of ribosomal protein mutants by mutation of the β subunit of RNA polymerase[41]. Further, the antibiotics neomycin and spectinomycin, which inhibit polypeptide chain elongation, relieve the inhibitory effect of ppGpp on rRNA synthesis[42], an effect which is also observed in mutant bacteria with a thermolabile protein synthesis elongation factor G (ref. 43).

In vitro two components of the translation machinery have been shown to affect RNA polymerase specificity. One is fmet $tRNA_f^{met}$ whose specific binding to *E. coli* polymerase holoenzyme is dependent on both formylation and the initiator tRNA moiety[44]. This charged tRNA alters polymerase activity, in particular promoting transcription of λp*lac* DNA and reducing rDNA transcription. A second effector is the elongation factor TuTs which decreases the transition temperature for rRNA synthesis on *E. coli* DNA while simultaneously increasing the sensitivity of transcription to ppGpp[15,30]. With φ80 d_3 DNA as template the factor sets rRNA synthesis as a level intermediate between the maximum level, observed at 5–10 μM ppGpp and the minimum level observed at 200 μM ppGpp (A.T., unpublished observations), and concomitantly increases su_3^+ tRNA synthesis[30]. Thus the pattern of transcription observed in the presence of EF-TuTs is that characteristic of the enzyme in a state which is maximally sensitive to ppGpp.

RNA polymerase and growth

The number of ribosomes a bacterium produces is in general approximately adjusted to the environment it grows in so that during exponential growth no more ribosomes are synthesised than can be engaged in protein synthesis. Thus the rate of protein production, and hence of growth, is balanced with the available resources. This correlation has led to the view that the level of ribosomes limits protein synthesis and thus control of ribosome production is a major facet of the control of growth[45]. At moderate to high growth rates the synthesis of the major transcriptional products required for ribosome biosynthesis, r-protein mRNA and rRNA, is coordinately regulated but at low growth rates this relation no longer holds, rRNA being relatively overproduced[46]. However, the synthesis of both products is under stringent control, both *in vivo*[47] and *in vitro*[34], suggesting that the mode of transcriptional regulation in the two cases, although not identical, is rather similar.

I have argued that an important aspect of the control of

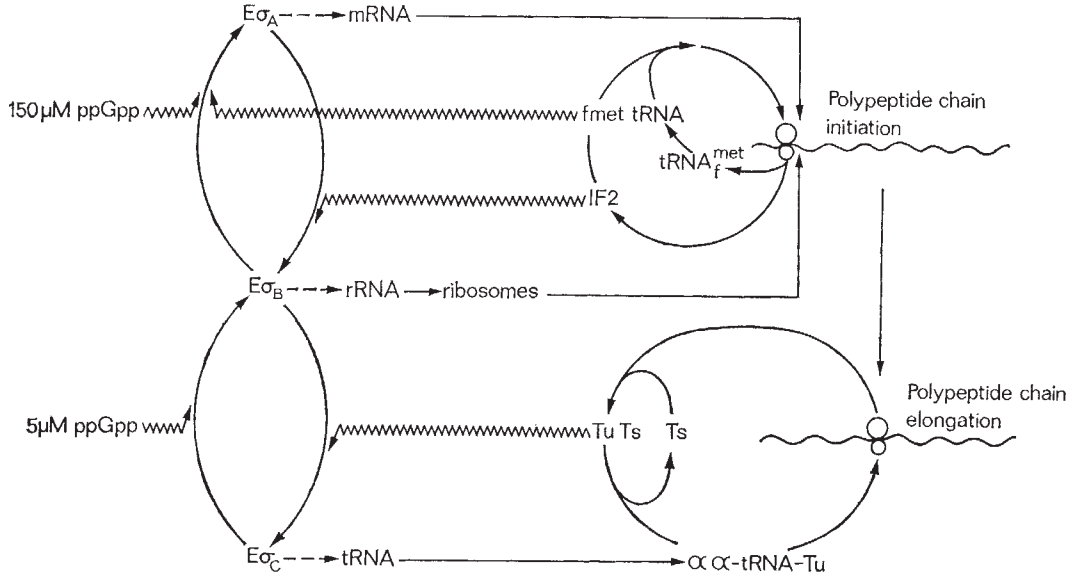


Fig. 3 A possible simplified scheme for the coupling of translation and transcriptional specificity. The types of RNA synthesised by the different forms of RNA polymerase represent the preferred specificity of the enzyme and do not represent absolute preferences. The term mRNA includes only certain mRNA species, for example, *lac* mRNA and T4 mRNA and excludes others, for example r-protein mRNA. The arrows indicating the direction of change in polymerase specificity do not imply any particular effecting mechanism.

rRNA synthesis is the response of RNA polymerase to intracellular signals. The question remains how such signals are integrated. Any molecular model for a control scheme is at present necessarily conjectural but must consider the logic of the control, the nature of the effectors and the dynamic balance between them.

One element in such a scheme is ppGpp acting as an effector of polymerase specificity in a manner similar to its *in vitro* function. Since the nucleotide probably alters the position of an equilibrium between specificity isomers the actual effect of ppGpp on transcription *in vivo* will depend on the relative free concentrations of ppGpp and of any effector which shifts the equilibrium in the reverse direction. Thus the mere accumulation of ppGpp need not invariably result in a shut off of stable RNA synthesis.

A second element is the alteration of the distribution between different specificity forms of RNA polymerase by the cycling factors of translation. Such effectors would signal the need for rRNA or for certain mRNA species or for even tRNA depending on which component of the translation machinery was momentarily limiting. For example free EF-TuTs might shift the polymerase towards tRNA production, assuming that the response of su_3^+ tRNA synthesis to effectors *in vitro* is representative of those tRNA species which are not cotranscribed with rRNA. Such a control constitutes a simple feedback loop since free EF-TuTs would be expected to accumulate when the supply of aminoacyl-tRNA was limited. Moreover the relatively high concentrations of EF-TuTs in the bacterial cell might be sufficient to override the low concentrations of ppGpp necessary to switch off su_3^+ tRNA synthesis by polymerase alone. Similarly fmet $tRNA_f^{met}$ could shift the pattern of transcription towards the synthesis of particular mRNA species. Again the logic is that of a simple feedback loop since fmet tRNA is consumed by the act of initiation of a polypeptide chain. The identity of the factor(s) maximising rRNA synthesis remains unknown[14]. It would seem logical, however, for rRNA production to be regulated by a factor involved in the initiation of protein synthesis. One candidate would be IF-2. The existence of several effectors regulating RNA synthesis implies that a mutation regulating in any one of the control elements would not eliminate entirely the synthesis of a particular RNA species but rather that such a change might only alter quantitatively the response to, for example, changes in growth medium. The number of bacterial proteins that interact with RNA polymerase is large[48] and thus the examples cited above possibly represent only a small fraction of the available control potential.

The data I have discussed relate to the *E. coli* holoenzyme in which the core polymerase is complexed with the σ subunit, a polypeptide of molecular weight ~90,000. In addition to σ, however, σ', a polypeptide of molecular weight ~56,000 of apparently similar function, is sometimes found complexed with core enzyme in place of σ[49]. In *B. subtilis* RNA polymerase is isolated exclusively in this form[50] while in *E. coli* σ' can be purified from ribosomes (R. Buckland and A. Travers, unpublished). The relative roles of σ and σ' *in vivo* are at present unknown and thus the possibility that the regulation of template selection by the σ'-containing enzyme differs from that of the σ-containing enzyme remains open.

The model outlined above envisages that the regulation of polymerase specificity is but one facet, albeit an important one, in the complex interplay of different modes of molecular control operating during normal bacterial growth. The transcribing enzyme complements the function of the highly specific DNA binding control elements for individual operons. In such a hierarchy, regulation of RNA polymerase specificity would be essentially a coarse control while fine control of particular transcripts would be provided by operon specific regulators.

Eukaryotic polymerases

To what extent are the principles of control of transcriptional specificity in prokaryotes applicable to eukaryotes? The transcriptional machinery of eukaryotes differs in at least one major respect from that of prokaryotes. In place of the single eukaryotic polymerase the nucleus of higher eukaryotes contains three structurally distinct enzyme types[51]. One, polymerase A, is found in the nucleolus and synthesises rRNA. A second, polymerase B, is located in the nucleoplasm where it synthesises hnRNA while the third, polymerase C, synthesises 5S and tRNA. These enzymes are all heteromultimers whose subunit structures, although more complex, bear strong resemblances to those of the bacterial polymerase. If the prokaryotic and eukaryotic enzymes are indeed homologues it would seem unlikely that the property of specificity modulation acquired by the prokaryotic polymerase would be entirely lost during the emergence of the eukaryotic line.

The separation of eukaryotic polymerases into three distinct structural types, each with apparently different functions, effectively releases the eukaryote from the prokaryotic restriction of balancing the use of a single enzyme. Such balancing is clearly necessary when the cell requires the simultaneous synthesis of two major classes of RNA, whose respective promoters are recognised by different conformations of the prokaryotic polymerase. The uncoupling in eukaryotes implies that each polymerase type would possess a relatively restricted initiation specificity, a view supported by the correlation of the template discrimination *in vitro* of the different enzymes with their presumed functions *in vivo*. The possibility thus remains that within the restricted range of promoter recognition of each enzyme, modulations of specificity could still result in the selective synthesis of different classes of RNA. In particular, for example, one polymerase might discriminate between promoters for the initiation of different mRNA species. A precedent for this exists in *E. coli* where the synthesis of certain mRNA species, for example those coding for ribosomal proteins and factors, is subject to stringent control[47] whereas the synthesis of other mRNA species, for example φ80 mRNA[10], is not. In eukaryotes such control might be particularly effective, for example, during the early stages of development.

If the eukaryotic polymerases are controlled what might their effectors be? Although ppGpp has been detected at low levels in cultures of *Dictyostelium*[52] this nucleotide has no apparent effect on the activity of fungal polymerases[53]. Other guanosine polyphosphates have, however, been isolated from organisms as diverse as the fungus, *Achyla*[54], and the crustacean *Artemia*[55,56]. The compounds are dinucleotides with guanosine residues connected by 5′–5′ polyphosphates. In addition, in some cases one of the 3′ positions is also phosphorylated with one or two phosphate residues. In *Achyla* the accumulation of these compounds is directly correlated with the rate of protein synthesis and thus they could arise by a capping of ppGpp, or more probably of its precursor pppGpp, in a manner analogous to the capping of mRNA[57]. Significantly these nucleotides strongly influence the activity of all three eukaryotic RNA polymerases. The response of the activity of these enzymes to increasing concentration of these nucleotides[53] bears strong similarities to the effect of ppGpp on the prokaryotic enzyme. The diguanosine nucleotides are thus candidates for one of the effectors of the RNA polymerases of lower eukaryotes. Nevertheless, here, as also in higher eukaryotes, the biological importance of the control of RNA polymerase activity remains an open question.

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articles

Perception of melodies

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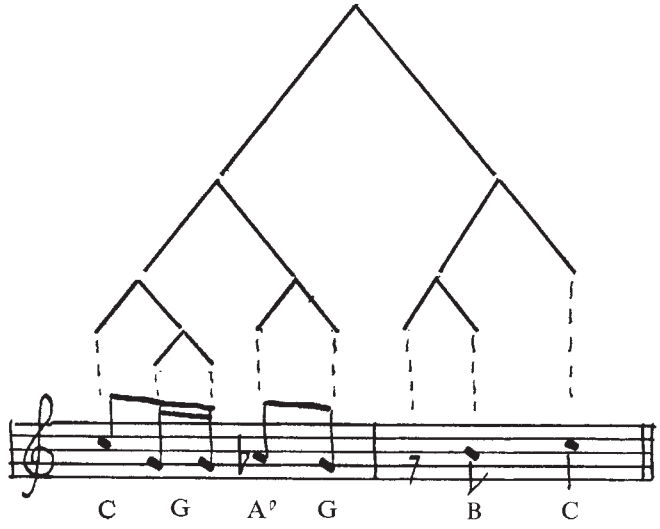
A computer program has been written which will transcribe a live performance of a classical melody into the equivalent of standard musical notation. It is intended to embody, in computational form, a psychological theory of how Western musicians perceive the rhythmic and tonal relationships between the notes of such melodies.

A SEARCHING test of practical musicianship is the 'aural test' in which the subject is required to write down, in standard, musical notation, a melody which he has never heard before. His transcription is not to be construed as a detailed record of the actual performance, which will inevitably be more or less out of time and out of tune, but as an indication of the rhythmic and tonal relations between the individual notes. How the musical listener perceives these relationships is a matter of some interest to the cognitive psychologist. In this paper I outline a theory of the perception of classical Western melodies, and describe a computer program, based on the theory, which displays, as best it can, the rhythmic and tonal relationships between the notes of a melody as played by a human performer on an organ console.

The basic premise of the theory is that in perceiving a melody the listener builds a conceptual structure representing the rhythmic groupings of the notes and the musical intervals between them. It is this structure which he commits to memory, and which subsequently enables him to recognise the tune, and to reproduce it in sound or in writing if he happens to be a skilled musician. A second premise is that much can be learned about the structural relationships in any ordinary piece of music from a study of its orthographic representation. Take, for example, the musical cliché notated in Fig. 1.

Fig. 1



The way in which the notes are rhythmically grouped is evident from the disposition of the bar lines and the "beams" linking the notes of the first bar. The rhythm is, in this case, a binary tree each terminal of which is a note or a rest, but more generally, such a tree may have ternary as well as binary nodes.

The tonal relations between the notes in Fig. 1 are also indicated by the symbolism, but more subtly. It is a common mistake to suppose that the position of a note on the five-line stave (and its prefix, if any) indicates merely the approximate pitch of the note—where it would be located on the keyboard. If that were true, an equally acceptable alternative to Figure 1 would be Fig. 2, in which the A♭ has been written as a G♯, with the same location on the keyboard. But a music student

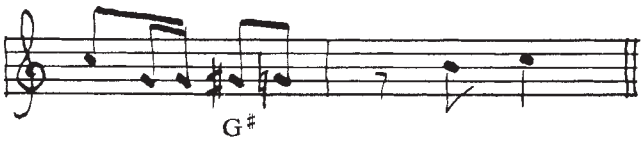


Fig. 2

who offered Fig. 2 as his transcription would lose marks for having misrepresented the tonal relation of the fourth note to its neighbours (though he could hardly be imagined not to have perceived it properly!).

The problems posed by melodic perception are not dissimilar to those which arise in the perception of speech. The distinction between the A♭ in Fig. 1 and the G♯ in Fig. 2 is analogous to the difference between the homophones "here" and "hear" in English; though these words sound exactly alike they are interpreted and spelt quite differently according to the context in which they are heard. Another problem in speech perception, which has its counterpart in the perception of melody, relates to the timing of successive acoustic events. The way in which the syllables of a poem are perceptually grouped into "feet" is largely unaffected by variations in rate of delivery, and the same applies to the rhythmic grouping of the notes of a melody. Notes which, on paper, are of equal length, will in a live performance be sounded at quite unequal intervals of time, particularly in an "expressive" performance. A change of metre from duplets to triplets can, nevertheless, usually be distinguished quite clearly from a mere quickening of tempo, in a reasonably competent performance. Previous programs for the automatic transcription of music have required the performer to maintain a fairly constant tempo[1,2]; but human listeners have no difficulty in discerning the rhythms of melodies played by performers who are free from this constraint.

The third premise of the theory is that the perception of rhythm and the perception of tonal relationships can be viewed as independent processes. This strong claim (which is not to be misunderstood as referring to the process of musical composition) may be weakly supported by two observations. First, that a given melodic sequence such as the ascending major scale will be heard as such by a Western musician regardless of the rhythm in which it is played. And conversely, that a "dotted" rhythm, for example, will be clearly recognisable for what it is, regardless of the musical intervals between the successive notes. To say this is not, of course, to deny that higher cognitive processes can and will operate on the "surface structure" generated by rhythmic and tonal perception, to reveal musically significant relations between the rhythm and the tonality. But one may reasonably suppose that such processes of musical appreciation can only begin when some structure has been created on which they can get to work.

Rhythm

One might imagine that to discern the rhythm of a melody the listener must be able to perceive differences in loudness between successive notes. This may be true on occasion but fails as a generalisation for two reasons. First of all, performers do not as a rule thump out every note which occurs on a beat or at the beginning of a bar; to do so would be as tiresome as to accent, in reading a poem, every syllable that occurred at the beginning of a foot. But more decisively, there are instruments such as the organ and harpsichord on which it is physically impossible for the performer to vary the acoustic intensity of each individual note; all he can control is the time of onset of the note and its temporal duration. It is nevertheless quite possible for a listener to perceive correctly the rhythm of a melody played on such an instrument; we conclude that temporal information alone is enough for the purpose, except in special circumstances.

The basic assumption underlying the rhythmic component of the program is that the first necessity in perceiving the rhythmic structure of a melody is to identify the time of occurrence of each "beat". Music in which the beat is irregular falls outside the scope of the theory, which therefore has nothing to say about the rhythmic perception of recitative or of music in which, for example, the beats alternate in length. The grouping of the beats into higher metrical units such as "bars" raises issues which have been discussed elsewhere[3,4]; the principal concern of the present study is with the manner in which each beat should be subdivided, and with the problem of keeping track of the beat through unforeseen changes in tempo.

In Western music by far the commonest subdivisions of the beat are into 2 and into 3 shorter metrical units; these in turn can be further subdivided into 2 or 3. Whether a beat, or a fraction of a beat, is perceived to be divided, depends, according to the theory, on whether or not it is interrupted by the onset of a note. What counts as an "interruption" is a matter of some delicacy, to which I shall return in a moment.

After such a process of division, and subdivision, every note will find itself at the beginning of an uninterrupted metrical unit. It is the relations between these metrical units which constitute the rhythm of the tune; the metrical units can be thought of as the nodes of a "tree" in which each non-terminal node has either two or three descendants. Every terminal node in the tree will eventually be attached either to a rest or to a note (which may be sounded or tied) in the manner of Fig. 1. The program does not actually draw such trees, nor print out a musical stave; it represents the rhythm in a nested bracketed notation. It also indicates the phrasing; if the offset of a note occurs earlier than half-way through its allotted time, or else appreciably before the end of that time, the note is marked "*stc*", standing for "*staccato*", or "*ten*" for "*tenuto*".

We now return to the question: what counts as an interruption? By what criterion could a listener judge whether the onset of a note occurs "during" the current metrical unit rather than "at" its beginning or its end? Plainly there must be some upper limit to the temporal discrepancies he can disregard, just as there is a lower limit to those he can detect. The former limit—the listener's "tolerance"—must obviously exceed the latter. It must be small enough to permit the structuring of rapid rhythmic figures, but large enough to allow a reasonable degree of flexibility to the tempo. In the program the tolerance can be preset to any desired value. Experiments with the program indicate that for reasonably careful performances a tolerance of about 10 cs meets both criteria, but that for more "expressive" performances, of relatively sedate melodies, a greater tolerance is needed if an obvious *rubato* is not to be misconstrued as a variation in rhythm.

In order to perceive the rhythm clearly a listener must, it is assumed, take account of the precise onset time of every note within any metrical unit in predicting when the unit could end. The rule eventually adopted for making such predictions was as follows: if, in the course of a binary metrical unit, a note which terminates the first subunit begins a little less than half way through, then the expected further duration of the unit is reduced in magnitude, to the mean of its original value and the value implied by the time of onset of the note in question.

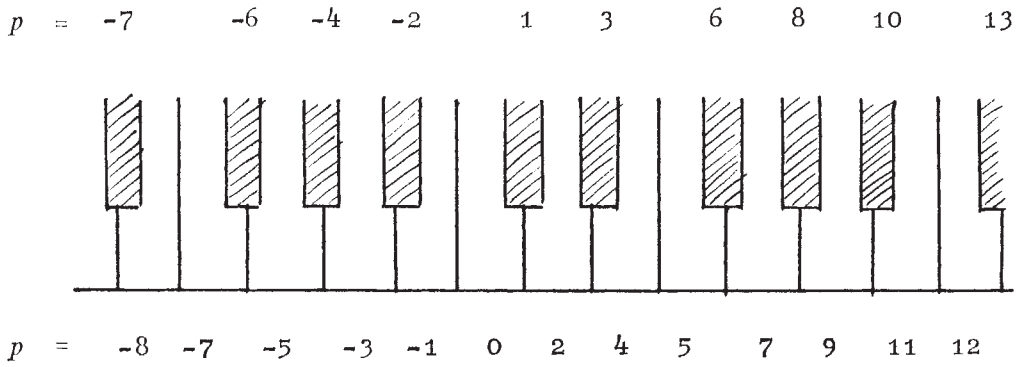


Fig. 3

Corresponding remarks apply, of course, in cases where the note is slightly late or the current metrical hypothesis assigns a ternary rather than a binary structure to the metrical unit in question. In fact the program also allows for the termination of a metrical unit, not by the actual onset of a note, but by the anticipated end of a lower metrical unit which is itself interrupted by the onset of one or more notes. Such procedures are, unfortunately, much more difficult to specify precisely in English than in a suitably designed programming language; but this fact only underlines the value of casting perceptual theories in computational form.

Finally, it is necessary to commit oneself, in writing such a program, to a view as to what counts as good perceptual evidence for a change in metre. It is here assumed that the listener initially expects a pure binary metre, but is prepared to change his mind at any level in the metrical hierarchy. The evidence for a change in metre may be of two kinds: that the current metre implies a "syncopation", in which the beginning of the next beat, or higher metrical unit, is not accompanied by the onset of a note; or that it implies a "distortion" in which an excessively large change of tempo is required to accommodate the current metrical hypothesis. Each of these outcomes represents a flouting of the listener's expectations and either may, according to the theory, lead him to change his opinion about the metre if the other possible division of the current metrical unit (ternary instead of binary, or vice versa) does not imply a distortion or a syncopation. Lastly, it is assumed that once having changed his mind the listener does not change it back again until he encounters positive evidence for doing so.

Tonality

In committing a melody to memory the listener must not only create a rhythmic structure of the kind depicted in Fig. 1; he must also identify the tonality of each note which is to be attached to it. This tonal information should ideally suffice, not only for the transcription of the melody into standard notation, but also for the purpose of evaluating the intonation of a performance on, say, the violin, which permits fine distinctions of pitch which cannot be made explicit on a keyboard instrument (see Fig. 3.)

To appreciate what is involved in this task it is necessary to formalise the classical theory of tonality, as developed by Rameau[5], Bosanquet[6], Helmholtz[7] and other writers. In the formal theory[8] every musical note is assigned coordinates (x, y, z) in a "tonal space" of three dimensions, corresponding to the perfect fifth, the major third and the octave, respectively. (The ideal frequency ratios of these intervals are 3/2, 5/4 and 2/1 respectively—involving the first three prime numbers—so that they are strictly incommensurable when not distorted by equal temperament.) Thus in Fig. 4, if the origin is taken as "middle C", the tonal coordinates of the various notes are as shown in the first three rows of the table. The following points may be noted: (1) It is the relative values of the coordinates (x, y, z), not their absolute values, which characterise the melodic sequence. Thus, increasing all the z values by 1 would merely put the melody up one octave. (2) The numerical values of the coordinates (x, y, z) are all small; this is evidently the result of having chosen middle C as origin. (3) The "position" p of each note on the keyboard, that is, its distance above middle C in keyboard semitones, is given by

$$p = 7x + 4y + 12z$$

so that there are arbitrarily many different notes (x, y, z) with the same value of p. (4) The conventional name of any note is determined not by its keyboard position p but by its "sharpness" q, defined as

$$q = x + 4y$$

The name N of the note is such that there are q sharps, or $-q$ flats, in the key signature of N major. Thus an "A" is a note with $q = 3$, and an "A♭" is one with $q = -4$.

x	0	1	0	1	.	1	0	(0)
y	0	0	-1	0	.	1	0	(2)
x	1	0	1	0	.	0	1	(0)
p	12	7	8	7	.	11	12	(8)
q	0	1	-4	1	.	5	0	(8)
N	C	G	A	G	.	B	C	(G)

Fig. 4

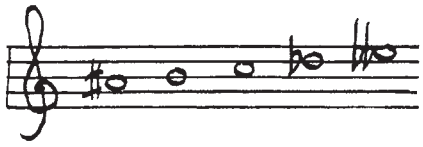


Fig. 5

The task of naming the notes of a melody played on the keyboard, or of notating them correctly on the five-line stave, therefore involves the apparently insoluble problem of determining the three coordinates (x, y, z) of each note from its keyboard position p, so as to be able to determine its sharpness q. The problem is analogous to the visual problem of adding an extra dimension to the 2-dimensional image of a three-dimensional scene, except that a keyboard performance of a melody supplies the listener with only one directly audible dimension, to which he must add two more to identify each note uniquely.

There is in fact a short cut to the solution of this problem, arising from the mathematical fact that a given choice of p severely restricts the range of possible values of q. A little simple arithmetic shows that q must differ from $7p$ by a multiple of 12. If, therefore, we can find independent grounds for limiting q to one of a fairly small set of values, we can determine it uniquely from the remainder upon division of p by 12. A survey of the published scores of classical melodies reveals[8] that the value of q (which can be directly determined from the notation) never changes from one note to the next by more than 11 units of sharpness. As a consequence, if δp is the "span" of any interval between two successive notes, then the "degree" δq of that interval is restricted to the following alternative values when δp lies in the range -11 to $+12$

$$\delta p = \begin{array}{cccccccccccc} 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 & 10 & 11 & 12 \\ \text{or } -11 & -10 & -9 & -8 & -7 & -6 & -5 & -4 & -3 & -2 & -1 & 0 \end{array}$$

implies that

$$\delta q = \begin{array}{ccccccccccccc} -5 & 2 & -3 & 4 & -1 & 6 & 1 & -4 & 3 & -2 & 5 & 0 \\ \text{or } 7 & -10 & 9 & -8 & 11 & -6 & -11 & 8 & -9 & 10 & -7 & \end{array}$$

By far the commonest intervals occurring in classical and traditional melodies are "diatonic" intervals, with $|\delta q| < 6$. "Chromatic" intervals, with $|\delta q| > 6$, and "diabolic" intervals, with $|\delta q| = 6$, are relatively rare. If they were nonexistent, the degree δq of each interval would be uniquely determined by its span δp, and one could infer the sharpness of each note in any melody from its position relative to its immediate predecessor. Such a "Markovian" theory of tonal perception would, as it happens, correctly predict the q values in Fig. 4. It would, however, fail dismally for melodies containing chromatic scales, because each keyboard semitone in such a scale would be assigned the same value of δq, and this would lead to absurd tonal interpretations such as that shown in Fig. 5.

An alternative, and musically much more plausible, hypothesis is that the listener identifies the sharpness of each note by placing it within a diatonic interval of the very first note (or perhaps an interval of degree 6 if the span indicates that the interval is diabolic). Such a rule would account equally well for the q values of the notes in Fig. 4. It would, furthermore, account very nicely for the sharpness of the notes in the theme of Bach's Musical Offering (Fig. 6)

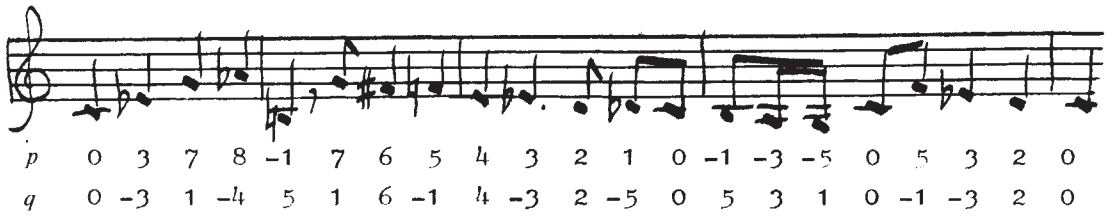


Fig. 6

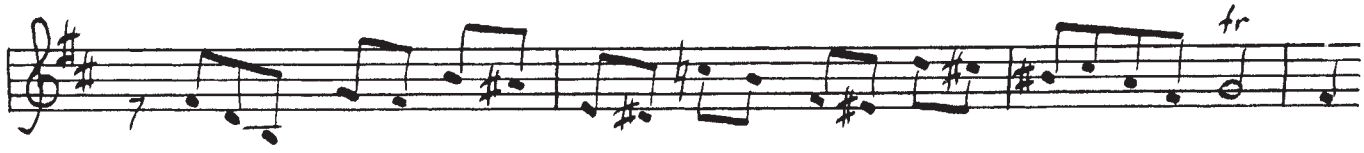
In this melody there are in fact four chromatic intervals—a diminished seventh between the 4th and 5th notes, of degree $\delta q = -9$, and three chromatic semitones, of degree -7. Such intervals could not, for obvious reasons, be correctly identified by the "Markovian" procedure.

It will not do, however, to assume that the first note is invariably the "keynote" to which every other note should be referred in order to determine its sharpness. First because melodies very often begin on notes other than the keynote, or "tonic", and second because the tonic may very well seem to change in the course of a melody, when we speak of a "modulation" having occurred.

A good example of an indisputable modulation is to be found in the subject of the B minor fugue in the first book of Bach's *Wohltemperierte Klavier* (see Fig. 7). The first three notes clearly establish the tonic as B ($q = 5$), and all the other notes in bars 1 and 2 are related to it by non-chromatic intervals ($|\delta q| < 7$). But the first note of bar 3, though in the same keyboard position as the C in bar 2, is notated differently. To have written it as a C would have produced the sequence C♯ C C♯, calling for two chromatic semitones in succession. But in unaccompanied classical melodies such an event never seems to occur, for the very good reason that if $X\ Y\ Z$ are three successive notes of a melody which, on paper, are separated by chromatic intervals $X\ Y$ and $Y\ Z$, then there is always an alternative, simpler, interpretation of the middle note Y which transforms both intervals into diatonic ones. Generally speaking, then, the tonal identity of a note cannot be finally established until the following note is heard. In Fig. 7 the offending note has become transformed into a B♯, making both the neighbouring intervals into diatonic semitones, of degree 5 and -5 rather than -7 and 7 respectively. But a B♯ is too far from the old tonic B to belong to its key, so that a modulation is perceived to occur to a new key, that of F♯, such that the value of δq for the new note B♯ is only 6, which is just close enough for comfort.

There seem to be other general restrictions upon the contexts in which chromatic intervals occur in classical melodies. The

Fig. 7



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: printlist(cliche);

[ 12 154 227]
[ 36 285 294]
[ 31 322 327]
[ 31 336 341]
[ 32 349 383]
[ 31 384 407]
[ 35 445 453]
[ 36 484 527]
```

a

Fig. 8

```
: 10->tolerance; notate(cliche);
```

b

```
[[[ 24 C STC] [[-5 G STC] [ 0 G STC]]] [[ 1 AB] [-1 G TEN]]]

[[[REST] [ 4 B STC]] [ 1 C TEN]]

:
```

c

most important of these relates to four-note sequences $W\ X\ Y\ Z$ in which the middle interval is chromatic. In such a sequence not only must both $W\ X$ and $Y\ Z$ be non-chromatic, but at least one of the intervals $W\ Y$ and $X\ Z$ must be diatonic. If the interpretations of W, X, Y and Z based on the current key violate this rule, then the tonality of the note Y is reinterpreted in such a way as to make $X\ Y$ a diatonic interval, and to force a modulation into a key to which Y belongs. As implied by what has been said, a note is regarded as belonging to a given key if its sharpness relative to the tonic lies in the range -5 to $+6$ inclusive.

Another rule which seems to be necessary in order to account for the notation of chromatic scales, particularly in music of the period following Bach, concerns the tonal interpretation of ascending semitones. If such a semitone ends on the note of a key whose sharpness relative to the tonic is 2, 3, 4 or 5, then its first note is to be assigned a relative sharpness of 7, 8, 9 or 10. Though this reassignment places the note outside the key, it does not by itself precipitate a modulation; if it did, then an ascending chromatic scale of any length would trigger a whole sequence of modulations into progressively sharper and sharper keys.

Two further rules are necessary, and sufficient, for determining the relative sharpnesses of the notes in most classical melodies. The first is a rule to the effect that for the purposes of establishing tonality one may conflate repeated notes, or notes separated by an octave (the second and third notes in Fig. 4 provide an example). The other rule, which is theoretically less satisfactory, is that the tonic may be determined from the first two notes, and that it will be either the first note itself or the note a fifth below it. This rule, and the absence of any more delicate tests of modulation than those already described, are undoubtedly the weakest links in the tonal section of the program.

Before I describe the program in detail, a few words of caution may be in order. First, the tonal rules outlined above must not be expected to apply to accompanied melodies, where the accompaniment supplies tonal information which may not be implicit in the melody itself. Nor must it be supposed that the rules necessarily hold for covertly polyphonic melodies in which, for example, alternate notes really belong to two different melodies. Further, the contextual constraints on chromatic intervals will often be violated at phrase boundaries, marked by rhythmically prominent rests, though this is not always the case. And finally, one must allow for the possibility that in a musical score a radical change of notation (such as occurs between the first and second sections of Chopin's *Raindrop Prelude*) does not signify a real change in tonality, but merely an "enharmonic change" designed to simplify the reader's task. Only if such qualifications are borne in mind can the program safely be used to indicate how a melody performed on the keyboard should be transcribed into conventional notation.

The program

The program accepts as input a list of sublists, each of which comprises three numbers. The first number is the keyboard position of the corresponding note and lies in the range 0 to 48, there being four octaves on the organ console. The second number is the time in centiseconds at which the note was depressed, and the third number indicates the time at which the note was released. The order of the notes on the list is the order of their times of onset. The list itself is generated from a live performance of a melody on an electronic organ connected, through an analogue-to-digital converter, to a high speed paper tape punch. The information on the paper tape is equivalent to the information which would be recorded on a player piano roll, and no more. The preprocessing of the paper tape is an entirely automatic matter, which simply involves constructing the above-mentioned list from the paper tape record and transferring it to disk storage.

The performer is required, by the present version of the program, to establish the initial tempo and the number of beats in a bar by prefacing his performance of the melody by a bar's worth of beats on some low note, which may conveniently be positioned an octave below the first note of the melody, so as not to prejudice the tonality.

The program itself is written in POP2, the high level programming language designed and developed in Edinburgh by Burstall and Popplestone[9]. It is relatively short, and is structured as follows. First, the list of sublists is converted into a list of records, each of which has a "slot" indicating the pitch, onset time and offset time of a particular note, and further slots which are to hold the span δp and the degree δq of the interval between the note and its predecessor. The keynote is then fixed by the positions of the first two notes, and the relative sharpnesses of all the notes are determined from their keyboard positions by an algorithm based on the theory of tonality

```
: printlist(tris);

[ 12 24 114]
[ 12 148 238]
[ 24 274 399]
[ 31 400 554]
[ 34 551 587]
[ 32 586 671]
[ 27 669 711]
[ 32 707 794]
[ 26 795 831]
[ 31 829 860]
[ 24 863 895]
[ 29 895 989]
[ 31 987 1021]
[ 29 1020 1145]
[ 27 1140 1242]
[ 26 1268 1282]
[ 24 1289 1298]
[ 22 1308 1320]
[ 29 1332 1452]
[ 26 1450 1495]
[ 22 1508 1517]
[ 21 1528 1536]
[ 20 1546 1556]
[ 27 1570 1696]
[ 24 1692 1734]
[ 20 1752 1762]
[ 19 1774 1782]
[ 18 1792 1808]
[ 26 1815 1930]
[ 29 1928 1934]
[ 27 1932 2062]
[ 26 2059 2188]
[ 25 2183 2446]
[ 24 2491 2628]
```

a

Fig. 9

```
: 13->tolerance; notate(tris);

[ 12 C][ 7 G]

[[[TIED G] [ 3 BB]] [-2 AB]][[[TIED AB] [-5 EB]] [ 5 AB]]

[[[TIED AB] [-6 D]] [[ 5 G] [-7 C]]][[ 5 F] [[TIED F] [ 2 G]]]

[-2 F][-2 EB TEN]

[[[-1 D] [-2 C STC] [-2 BB]] [ 7 F]][[TIED F] [-3 D TEN]]

[[[-4 BB STC] [-1 A STC] [-1 AB]] [ 7 EB]][[TIED EB] [-3 C TEN]]

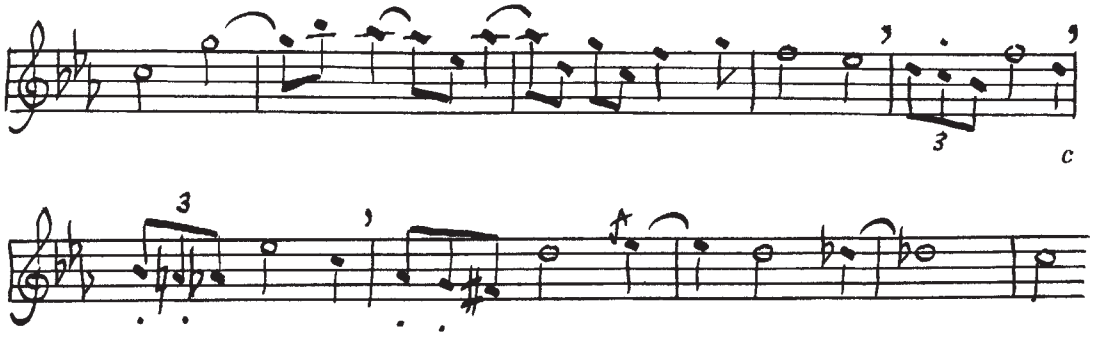
[[[-4 AB STC] [-1 G STC] [-1 FS]] [ 8 D]][[TIED D] [ 3 F -2 EB]]

[[TIED EB] [-1 D]][[TIED D] [-1 DB]]

[TIED DB][TIED DB]

[-1 C]
```

b



c

outlined in the previous section. The next stage is a rhythmic analysis (which could have been carried out first, as it is indifferent to the results of the tonal routines). Each beat is examined in turn, by a combination of "top down" and "bottom up" analysis in which the time of onset of each note is used both for establishing the structure of the rhythmic hierarchy and for correcting the estimated tempo. In the course of this analysis the time of offset of each note is used for determining how the note was phrased.

The final stage in the operation of the program corresponds to the exercise of musical literacy; it consists of displaying, on paper, the essential features of the structure created by the rhythmic and tonal analyses as a sequence of nested lists of symbols. The innermost symbols name the individual notes as, for example, 'D' (D natural), 'DS' (D sharp), or 'DB' (D flat); the word REST is self-explanatory. Each name is preceded by either the word TIED if the note is tied to its predecessor or a number indicating the span (not the degree, which is implicit in the name of the note) of the interval from the preceding note; this is needed for identifying the octave in which the note occurs. Finally, a note which is not tied may be followed by the abbreviation STC or TEN indicating that the note was played *staccato* or *tenuto*; the absence of either abbreviation implies that the note was played *legato*.

Figures 8, 9 and 10 provide examples of the program's performance. Each figure indicates (*a*) the "raw" input, in which each set of three numbers gives the keyboard position of a note and its times of onset and offset in centiseconds, (*b*) the output generated by the program from the input (*a*), and (*c*) the result of transcribing the output (*b*) by hand into ordinary stave notation.

The performance of the tune shown in Fig. 8 was prefaced by a single low C, and the time between the onset of this note and the next was arbitrarily taken as a "minim" in adopting the note values indicated in (*c*); it will be noted that in (*b*) the outermost brackets enclose a minim's worth of notes. The interpretation (*b*) was obtained from the input (*a*) with a tolerance of 10 cs; with a tolerance of 15 cs the program would assign the two semiquavers to the same node. It is worth noting that the actual times of onset of the first four quaver

```
: printlist(stan);

[ 19 148 190]
[ 31 280 287]
[ 29 302 309]
[ 27 322 329]
[ 34 347 466]
[ 31 474 518]
[ 27 538 548]
[ 26 559 566]
[ 25 578 586]
[ 33 605 648]
[ 30 646 657]
[ 26 669 678]
[ 25 687 696]
[ 24 707 714]
[ 32 729 760]
[ 29 769 777]
[ 25 791 801]
[ 24 811 820]
[ 23 830 839]
[ 31 856 987]
[ 27 986 1027]
[ 24 1049 1054]
[ 23 1068 1075]
[ 22 1087 1096]
[ 29 1111 1153]
[ 26 1152 1157]
[ 22 1174 1183]
[ 21 1194 1202]
[ 20 1211 1220]
[ 27 1232 1270]
[ 24 1272 1279]
[ 20 1295 1304]
[ 19 1316 1325]
[ 18 1336 1348]
[ 26 1360 1619]
```

a

Fig. 10

```
: 13->tolerance; notate(stan);

[[[ 12 G STC] [-2 F STC] [-2 EB STC]] [ 7 BB]]

[[TIED BB] [-3 G TEN]]

[[[-4 EB STC] [-1 D STC] [-1 CS STC]] [[ 8 A] [TIED A] [-3 FS]]]

[[[-4 D STC] [-1 CS STC] [-1 C STC]] [[ 8 AB] [REST] [-3 F STC]]]

[[[-4 DB STC] [-1 C STC] [-1 B STC]] [ 8 G]]

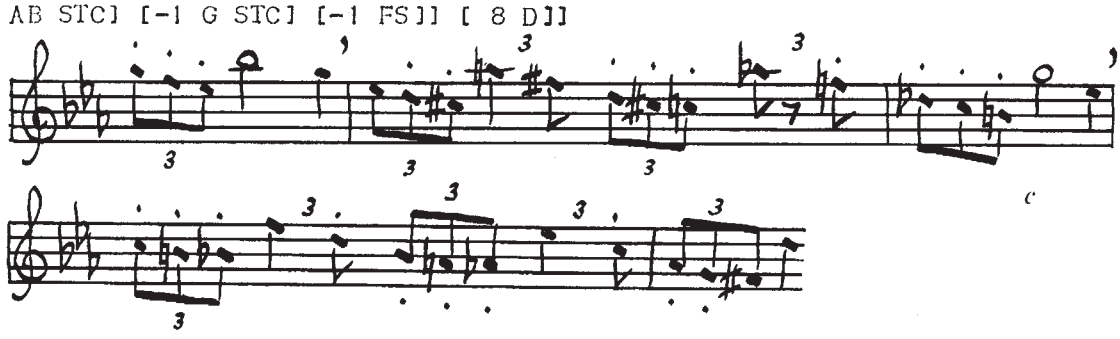
[[TIED G] [-4 EB TEN]]

[[[[-3 C STC] [-1 B STC]] [-1 BB STC]] [[ 7 F] [TIED F] [-3 D STC]]]

[[[-4 BB STC] [-1 A STC] [-1 AB STC]] [[ 7 EB] [TIED EB] [-3 C STC]]]

[[[-4 AB STC] [-1 G STC] [-1 FS]] [ 8 D]]
```

b



c

units differed in the performance by 37, 27 and 35 cs respectively, the separation between the last two notes being 39 cs. The considerable discrepancy between these numbers clearly illustrates the acute difficulty which would confront any attempt to determine the rhythm without taking account of its hierarchical structure.

Figure 9 shows how the program handled a performance of part of the long cor anglais solo from the prelude to Act III of Wagner's *Tristan und Isolde*. This example is interesting in two particular respects. First, it involves the perception of a change from a binary to a ternary metre in the fifth bar; and secondly, the published score indicates a grace note at the end of the seventh bar, to which it would be inappropriate to assign a separate place in the rhythmic structure. The program's output agrees fully with the score in its rhythmic and tonal indications; there are slight discrepancies in the marks of phrasing—Wagner marked all the triplet quavers as staccato—but for this the performer is clearly to blame, not the program.

Finally, Fig. 10 illustrates the program's handling of a later section of the same melody (prefaced, this time, by only one "cue" note, which is why each line of the output contains only one, not two, minim units). Again the rhythm is correctly represented, though there are minor discrepancies in phrasing. As for the tonality, the main point to note is the perceptual problem presented by the rapid succession of modulations beginning in the second bar. There is, nevertheless, only one note to which the program assigns a tonality at variance with that indicated by Wagner: he wrote the second C♯ in the second bar as a D♭.

Conclusions

The domain of competence of the program is, of course, very restricted: it cannot be expected to reveal significant tonal or rhythmic relations between the notes of "atonal" or "arhythmic" melodies, for example. But the perceptual theory on which it is based does seem worthy of serious consideration, in that up to the present time no detailed suggestions seem to have

been offered as to how a listener builds an internal representation of a melody from a live performance. The most significant rhythmic hypothesis in the theory is that the rhythm of a melody is conceptualised as a structural hierarchy, and that the onset of each note provides important predictive information about the time of onset of the following note at every level in the hierarchy. The hypotheses underlying the tonal section of the program are presumably limited in application to the kind of music that has been developed in the West; but for such music one conclusion at least seems secure, namely that the tonality of any note cannot in general be established unambiguously until the following note has been heard. It is perhaps surprising that such a limited amount of context should usually suffice for the purpose, but it should be remembered that it is really the key of the melody which creates the tonal context in the first place.

It seems altogether possible that the principles of operation of the program's rhythmic component will apply to other temporal processes such as the perception of speech.

A full account of the program and of the underlying theory is to be published elsewhere.

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New evidence on the origin of rotation measures in extragalactic radio sources

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Martine Simard-Normandin

David Dunlap Observatory, University of Toronto

The causes of the Faraday rotation in extragalactic radio sources have been reinvestigated in the light of new polarisation data which extend to large redshifts. Our results show convincingly that at nearly all galactic latitudes, the large rotation measures are generated outside of the Milky Way. Furthermore, any contribution from the intergalactic medium must be small when compared with the Faraday rotation generated at or in the extragalactic sources themselves. Having rotation measures for 108 QSOs of which 48 have redshift (z) *greater than unity, we have been able to place the lowest and most reliable upper limits on any contribution from an intergalactic magneto-ionic medium at early cosmological epochs. The data show promise that it should soon be possible to ascertain whether or not the density of intergalactic ionised gas follows the cosmological scale factor.*

THE Faraday rotation of radio waves from extragalactic sources has three possible origins: (1) a 'local' contribution in the Milky Way; (2) a possible contribution from intervening intergalactic material and (3) a contribution from the sources themselves. (We shall ignore ionospheric Faraday rotation, which is very small and can easily be removed.) The fact that there is a galactic contribution is well established, since the rotation measures (*RM*) show systematic variations when plotted on galactic co-ordinates[1-4]. Less clear, however, is whether the effects (2) and (3) are discernable, and there are conflicting claims in the literature[5-8] as to whether any intergalactic component can be seen. The source component (3) has hitherto been thought to be small[1-3].

In an effort to improve the situation we have measured linear polarisations at one or more wavelengths for a large number of QSOs and radio galaxies (refs 9, 10 and P. P. Kronberg, unpublished). After combining these data with other recent measurements[11,12] and other previously published polarisation data, we have computed rotation measures for 450 extragalactic sources. These include 108 QSOs of known redshift, of which 48 have a redshift $z > 1$—many more than the number previously in this category. Using the new larger data sample, we re-examine the *RM* with a particular view to reassessing the relative contributions of factors (1), (2) and (3). Of particular interest is whether any effect of an intergalactic medium (2) can be seen, since the detection of intergalactic gas by its effect on Faraday rotation of QSOs would have important cosmological significance.

Comparison of the galactic and extragalactic contributions

Figure 1 shows histograms of the observed *RMs* for all sources (radio galaxies, QSOs and unidentified) separately for (*a*) $|b| > 30°$, where the galactic contribution should be small, and for (*b*) $|b| \leqslant 30°$. Figure 1(*a*) shows that there is a clear separation into two populations; a narrow peak centred on $RM \simeq 0$ rad m^{-2} and not resolved by the bin width of 50 rad m^{-2}, and a second, broad distribution extending more or less symmetrically to approximately $\pm 1{,}000$ rad m^{-2}. For sources having $|b| < 30°$, the broad and symmetric group is still present, in fact the fraction of sources showing $|RM| > 200$ rad m^{-2} is virtually the same in (*a*) and (*b*)—34% and 35% respectively. There is a striking difference, however, between the distributions near $RM = 0$. What is a narrow peak of half-width ~ 45 rad m^{-2} in Fig. 1*a* (see inset) appears

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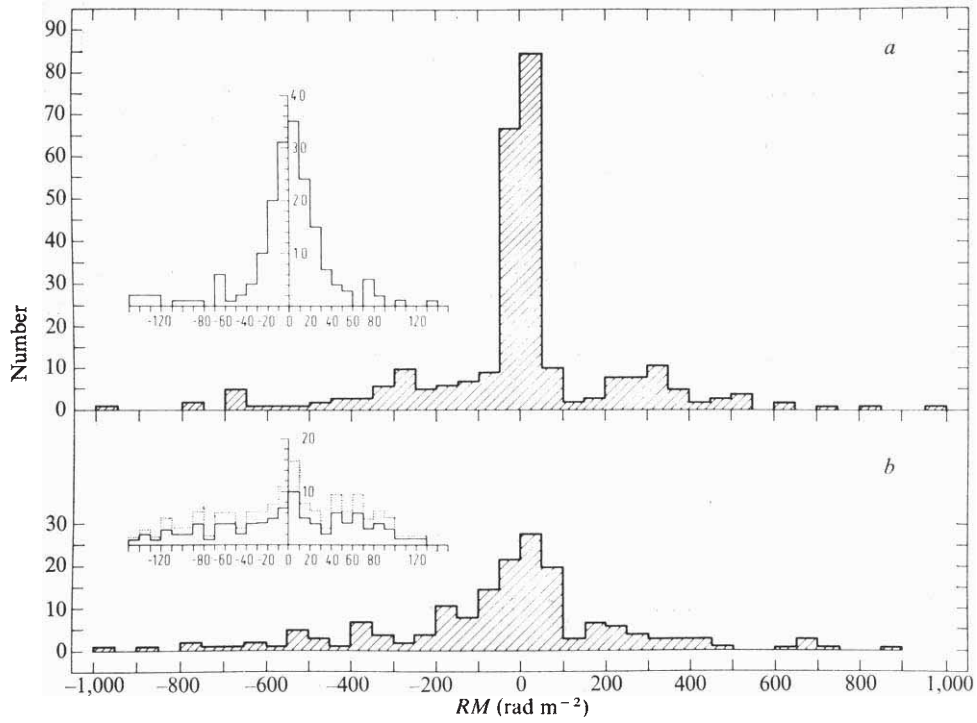

Fig. 1 Histograms showing the distribution of the rotation measures for (*a*) $|b| > 30°$ (275 sources and (*b*) $|b| < 30°$ (175 sources). The insets show the central peaks with intervals of 10 rad m^{-2}. The dotted profile in (*b*) shows the same distribution scaled by 275/175 for comparision with that in (*a*).

as distinctly broader distribution, with half-width ~120 rad m^{-2} for the low-latitude sources. Again comparing distributions (*a*) and (*b*) in Fig. 1 it is evident that sources with very low rotation measures $|RM| \lesssim 20$ rad m^{-2} occur nearly exclusively at the higher galactic latitudes ($|b| \gtrsim 30°$), whereas those with very large rotation measures occur with equal probability everywhere in the sky. The comparison between Fig. 1(*a*) and (*b*) is revealing, and enables us to draw some new and interesting conclusions. These are: firstly, the very large ($\gtrsim 200$ rad m^{-2}) values of *RM* are, on average, not caused by the Galaxy, since the fraction of sources with $|RM| > 200$ is independent of path length through the Galaxy. A two-dimensional galactic co-ordinate plot of the rotation measures (not shown) confirms that there is no large-scale order in Fig. 1*b* for the high *RM* sources, and that the large source-to-source variations of *RM* are just as large at high as at low galactic latitudes, and second, there is a small subset (~25% of the sources in our sample of 450) which have a very low intrinsic *RM* ($\lesssim 10$ rad m^{-2}). These form the narrow peak in Fig. 1(*a*) which, presumably, would be still narrower if we were to remove accurately the galactic component of *RM* at high latitudes.

The first conclusion is new, and contradicts the widespread belief based on earlier data[1-3] that most of the Faraday rotation was galactic. Our data show that the galactic component of *RM* must seldom be $\gtrsim 60$ rad m^{-2} for $|b| > 10°$. Because of various selection effects, there are very few *RM* at $|b| \lesssim 5°$ where the galactic rotation measure may be systematically $\gtrsim 200$ rad m^{-2}. Thus, our first conclusion may not apply to sources which lie very close to the galactic plane, but this question does not affect the discussion of the extragalactic contribution. We shall cite further evidence below which suggests that the bulk of the large *RM* is generated in or near the sources themselves.

Having established that the Galactic rotation measure is < 200 rad m^{-2}, except perhaps at very low latitudes, we attempted to estimate the extragalactic rotation measure of all sources with known redshift—108 QSOs and 90 radio galaxies. To do this we define the galactic rotation measure (*GRM*) to be the average *RM* within a 20° diameter circle centred on the QSO, for all other sources having $|RM| < 150$ rad m^{-2}. By eliminating sources with $|RM| > 150$ rad m^{-2} we tend to eliminate sources whose *RM* is largely extragalactic. It is the residual rotation measure ($RRM = RM - GRM$) derived in this way which we analyse further below.

The distribution of *RRM* for QSOs and radio galaxies

Figure 2 shows the distributions of *RRM* for 118 sources having $z \geqslant 0.2$ for five redshift intervals. Note that the 48 sources having $z > 1$ are nearly equally divided among the three largest redshift intervals. If the *RRM* calculated for each source

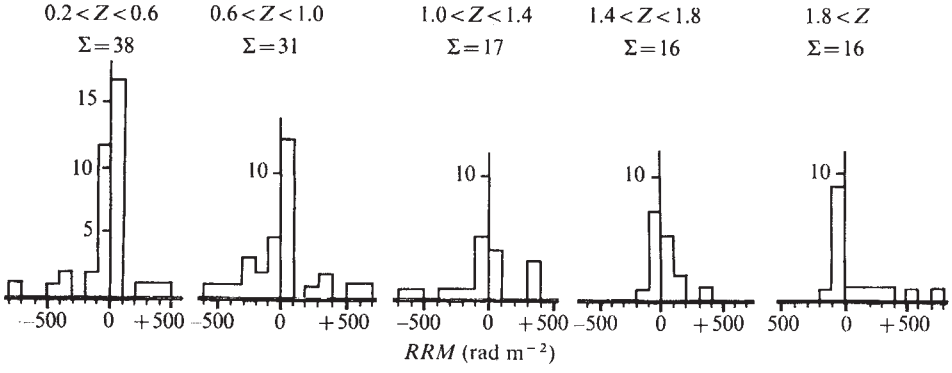

Fig. 2 Histograms showing the distribution of the residual rotation measures (*RRM*) for 5 equal redshift intervals beginning at $z = 0.2$. The formal variances are (from left to right): 4.0, 5.5, 6.6, 1.5 and 5.7 × 10^4 rad^2 m^{-4}.

were a true measure of the extragalactic component of its rotation measure, and if there were no intervening intergalactic material which contributes to the *RM*, we would attribute the observed variances solely to differences in internal Faraday rotations in the source themselves. If the internal Faraday rotation within the sources is independent of redshift (and assuming the emission line redshift also to be that of the radio emission) the variance should then decrease as $(1+z)^{-4}$. Figure 2 shows that this is clearly not the case: within the errors, the variance of *RRM* is largely independent of redshift for the 118 sources (101 QSOs) having $z > 0.2$. We also computed the *RRM* of a control sample of 90 radio galaxies with known redshift by the same method and plotted the variance over their (much smaller) range of z. There is likewise no systematic variation over the range $z \lesssim 0.4$, and the variances are also in the range $(4\text{–}7)\times 10^4$ rad^2 m^{-4} which is indistinguishably different from that for the QSOs.

We repeated the variance calculations for the same bin intervals and populations as in Fig. 2, but this time using different randomly-mixed redshifts for the sources. The variance is in the range $(5\text{–}6)\times 10^4$ rad^2 m^{-4}, and shows a slight tendency to increase at low z where there are more sources in the bin—an effect of a few anomalously large rotation measures which are more likely to fall in the low redshift bins since these have the largest populations. As a further test we recalculated the variances using 'observed' (*RM*) rather than the residual (*RRM*) rotation measures, and the average variance is only marginally greater, which is consistent with our expectation based on the results of Fig. 1.

We can conclude from this that the rotation measures have a variance $V \sim 5\times 10^4$ rad^2 m^{-4} even after attempting to remove the galactic contribution. This noise effect is not substantially reduced if we eliminate all sources close to the galactic plane ($|b^{11}| < 30°$), where the galactic contribution is larger. The fact that the scatter is of the same order of magnitude for the (low redshift) sample of radio galaxy *RRM* as for the QSOs suggests that the large source to source variations are not a sensitive function of intergalactic path length. The overwhelming contribution to large *RM* must therefore come largely from the sources themselves. This fact was previously surmised by Reinhardt[13], and it is also consistent with the observed depolarisation rates in many extragalactic sources, which often imply a differential Faraday rotation within the sources of several hundreds of rad m^{-2}.

It is now appropriate to consider whether some other source parameter correlates with *RM* (or *RRM*), which might enable a better statistical isolation of the intergalactic contribution. If so, we could improve the sensitivity of our test for variations of $V(z)$ in Fig. 2, which are overwhelmed by the scatter from *RM* generated at or in the sources themselves. For most of the QSOs we also have measurements of $\lambda_{1/2}$, the wavelength at which the linear polarisation falls to half its maximum value. This depolarisation is most likely caused by differential Faraday rotation within the sources themselves, and if we further assume the sources to have a uniform mixture of thermal and relativistic electrons the rotation measure which is intrinsic to the source, (*IRM*), can be related to $\lambda_{1/2}$ by

$$IRM = k\lambda_{1/2}^{-2} \text{ rad m}^{-2} \qquad (1)$$

where the factor k is $\simeq 1(\lambda_{1/2}$ in (m)) for most simple geometrical shapes which have an aligned internal magnetic field[14]. Obviously we do not expect the sources to behave as simple uniform mixtures of relativistic and thermal electrons in a perfectly homogeneous magnetic field, as $k = 1$ in equation (1) implies, but it is interesting to see if some statistical correlation exists. A plot of *RRM* against $\lambda_{1/2}^{-2}$ (Fig. 3) shows that there is in fact little correlation between depolarisation rate and rotation measure.

Figure 4 shows the distributions of the calculated values of *IRM* for the same redshift intervals as in Fig. 2 for the QSO sample. There is a possible tendency for the widths of the

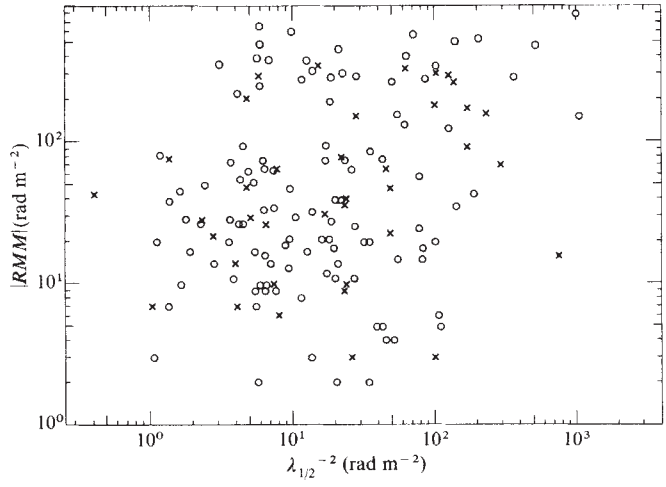

Fig. 3 A plot of *RRM* against $\lambda_{1/2}^{-2}$. o = $z < 1.0$; × = $z \geqslant 1.0$.

RRM distributions in Fig. 2 to reflect those of $\lambda_{1/2}^{-2}$ in Fig. 4, but there is still too little data for us to conclude that there is a statistical dependence of V on $\lambda_{1/2}^{-2}$, particularly in view of the poor correlation in Fig. 3. If we were to conclude that the variances ($V(z)$) merely reflected the *IRM* of the sources we could nonetheless still establish an upper limit to the contribution of an intergalactic medium to the dispersion in *RRM*, since any variation of $V(z)$ from an intergalactic magneto-ionic medium would be superimposed on the effect of the varying scatter in the *IRM*. We also tested for the effect of varying populations of radio spectral index, $\alpha(S\nu \propto \nu^{\alpha})$, with redshift. After splitting the QSOs according to $\alpha \lesssim -0.5$, we found no significant difference in the variances for each population, and conclude that the scatter in *RRM* in the present sample is quite insensitive to variations in radio spectral index.

These investigations show that in the absence of more and better data, a fairly large scatter in the *RM* values of QSOs with large z cannot be removed, and any contribution by Faraday-rotating intergalactic clouds would have to be $\gtrsim 100$ rad m^{-2} in the observer's frame to have a detectable effect on the formal variances. If, however, a substantial subgroup of sources with $RRM \simeq 0$ in fact persists out to the largest redshifts (analagous to the sharp peak near $RM = 0$ in Fig. 1) this would provide powerful evidence for the absence of any intergalactic gas. There is some suggestion that a narrow peak near $RRM = 0$ does persist out to $z \simeq 2$ (Fig. 2), but our sample of highly redshifted objects is not quite large enough to provide a firm answer to this question.

Limits to the contribution from an intergalactic medium

The fact that we find no detectable increase in the variance $V(z)$ of the *RRM*s with redshift (Fig. 2) enables us to put an upper limit on any contribution caused by intervening intergalactic clouds of magnetoionic material. If the ion density of the intergalactic clouds follows the universal scale factor and the (randomly oriented) magnetic field is frozen in, then

$$n(z) \propto (1+z)^3 \qquad (2)$$

$$|B|(z) \propto (1+z)^2 \qquad (3)$$

and the increase in $\langle n|B_{\parallel}|\rangle$ with redshift overwhelms the 'watering-down' effect due to the Doppler shift[15,6] ($\alpha(1+z)^{-2}$) in which case the variance should increase with redshift. For these assumptions the sensitivity of the *RM* data to an intergalactic medium increases as the source sample extends to greater redshift. For our sample, of which 48 sources have $1 < z < 2.8$, we can infer the following upper limit to an

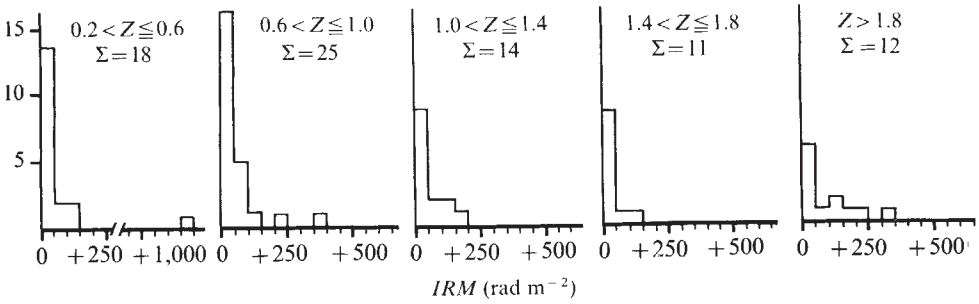

Fig. 4 Distributions of the intrinsic rotation measures of QSOs (in the observer's frame) as calculated from the assumption that $IRM = \lambda_{1/2}^{-2}$ and using the same redshift intervals as in Fig. 2. The samples here are slightly smaller than in Fig. 2, since a few sources do not have well-determined $\lambda_{1/2}$ values.

intergalactic medium in a Friedmann universe with $q_0 = 0.5$ and $H_0 = 75\ \mathrm{km\ s^{-1}\ Mpc^{-1}}$

$$n_0|B_{\parallel 0}| \lesssim \frac{1.1\times 10^{-13}}{l_0^{1/2}}\ \mathrm{gauss\ cm^{-3}}$$

where $n_0|B_{\parallel_0}|$ and l_0 are the mean values for the present epoch (averaged over many cells) of electron density, line of sight component of magnetic field, and cell size (Mpc) respectively. The cells are assumed contiguous. If we assume $B_{\parallel 0} = 10^{-8}$ gauss, then the corresponding density is 1.8 times the mean density

$$\left(\bar{\rho}_0 = \frac{3}{4\pi} q_0 \frac{H_0^2}{G}\right)$$

for this model of the Universe. This is not much above the mean density $\bar{\rho}_0$ and shows that with more data it should soon be possible to use rotation measure data to place useful limits on certain combinations of model universe and intergalactic medium parameters.

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Muon catalysed fusion for pellet ignition

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We describe here a new method for achieving controlled thermonuclear fusion which appears to give a better overall energy output over input ratio than for laser driven fusion.

THE idea is to use muon catalysed fusion reactions, which can take place at a temperature lower than that required for ordinary fusion reactions, to provide the energy needed for the ignition of ordinary fusion reactions in an inertially confined deuterium–tritium pellet. The pellet is first compressed to $\sim 10^3$ times solid density and preheated to $\sim 10^3$ eV by a relatively low power laser or electron beam acting as a prepulse. This is to enhance both the catalytic and ordinary fusion reaction rates. Simultaneously, or a short time beforehand, a pulse of muons (probably $> 10^{10}$ in < 1 ns) is injected into the pellet. The muon energy distribution is selected such that most of the muons are deposited in the core of the pellet. The distance stopped and the moderation time can be obtained as a function of the muon incident energy[1]. For example 1-MeV muons will be stopped in 10^{-3} cm in 10^{-11} s.

Basic reaction

When stopped, a muon cascades through the atomic energy levels and enters a muonic Bohr orbit about the nucleus (here a deuteron or triton). Since the muon is 205 times heavier than the electron its Bohr orbit (mean radius 2.6×10^{-11} cm) is very close to the nucleus. The orbiting muon thus effectively screens off the Coulomb potential of the nucleus. The muon is, moreover, polarised by an incident ion, resulting in further reduction of the Gamow barrier penetration width (Fig. 1 inset) and this greatly increases the probability of the fusion reactions which can be written as

$$\left.\begin{array}{l}\mu t + d^+\\ \mu d + t^+\end{array}\right\} \rightarrow \alpha(3.6\ \mathrm{MeV}) + n(14\ \mathrm{MeV}) + \mu \qquad (1)$$

In addition to the usual 3.6-MeV α particle and 14-MeV neutron, a muon with negligible energy is also ejected. The free muon is again captured by a deuteron or triton and the cycle is repeated until it is captured by a helium double ion, in effectively another branch of the reaction (1)

$$\mu t + d^+ \rightarrow \mu He^{4+} + n \qquad (2)$$

It is then unavailable to the catalytic fusion chain unless it is stripped off in the slowing down of the helium ion.

The branching ratio between reactions (1) and (2) is 100:1,

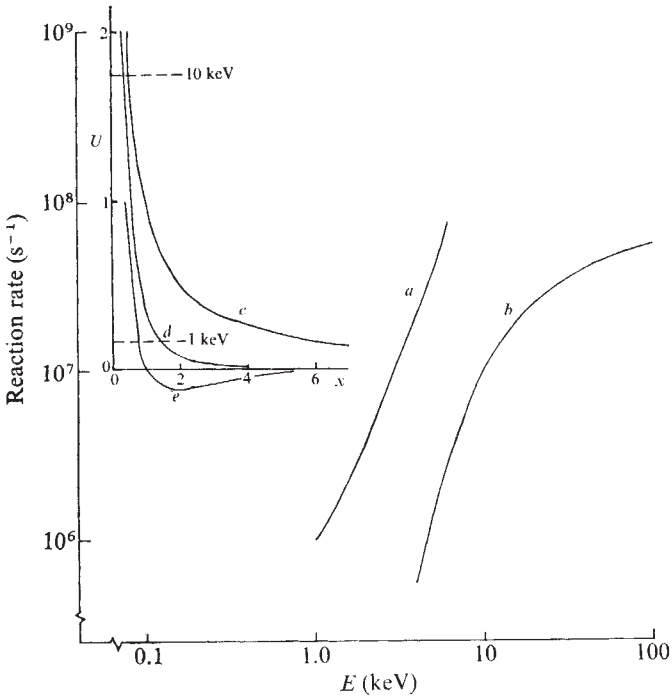

Fig. 1 Reaction rates of muon catalysed 'in flight' dt fusion reactions at solid dt density. *A*, . . . Muon catalysed reaction; *B*, . . . ordinary fusion reaction.

Inset: Potential distribution of muonic hydrogen atom and its polarisation by a positively charged nucleus. *C* . . . $U(x) = x^{-1}$; *D* . . . $U(x) = \exp(-x)/x$ (screened Coulomb potential); *E* . . . $U(x) = \frac{1}{x} - \frac{1.5}{(1+0.127x)^4}$ (Morse potential);

$$x = r/a,\ a = \text{muonic Bohr radius},\ U = \frac{E}{(e^2/a)}$$

which is also the catalytic chain ratio unless the pellet core confinement time is too short for the chain to be completed. Inertia confinement times are typically $\sim 10^{-9}$ s or shorter so that the muon which has a mean half life of 2×10^{-6} s is stable in our time scales.

Mechanism

Muon catalysed fusion reactions were initially studied by Frank[2] and Alvarez *et al.*[3] and put on a firm theoretical basis by Jackson[4], Belyaev *et al.*[5], Zel'dovich and Gershtein[6] and Carter[7]. One important conclusion is that the fusion reaction (1) in the cold medium, requires first the formation of the molecular ion $d\mu t^+$. The reaction rate is therefore controlled by the rate of formation of the molecular ion, and is therefore slow. In our scheme we propose to bypass this stage by preionising the electronic atoms.

The reaction rate, at constant density, then, is the product of two probabilities: the probability P_1 for barrier penetration and the probability P_2 of decay of the compound nucleus μdt into the right channel. $P_1(E)$, as a function of the energy, E, of the incident nucleus, has been computed by solving Schrödinger's equation to obtain the ratio of the wave amplitudes at the nucleus radius and at a large distance from it, using the Morse potential distribution, which is an approximation of the polarised screened Coulomb potential (Fig. 1 inset). For energies <10 keV, $P_2(E)$ is not known. There is, however, a resonance 'near zero energy'[8] and it is likely that P_2 is greater than the 2×10^{-14} cm^3 s^{-1} given by Jackson[4] which is roughly valid for an energy between 10 keV and 100 keV. Assuming $P_2 = 2\times10^{-14}$ cm^3 s^{-1}, the reaction rates for the reaction (1) at a solid density of 5×10^{22} cm^{-3} is given in Fig. 1, together with the ordinary fusion reaction rates.

Rates

To find the number of particles undergoing fusion reactions, it is not enough to know the reaction rates, since the muonic atom may be dissociated ('ionised') in certain conditions of temperature and density. Moreover, we require the pellet to be sufficiently ionised with respect to the electrons, that is for the reactions

$$\mu + t^+ \rightleftharpoons \mu t \text{ or } \mu + d^+ \rightleftharpoons \mu d$$
$$e + t^+ \rightleftharpoons et \text{ or } e + d^+ \rightleftharpoons ed \qquad (3)$$

we require

$$\alpha_{\mu t} \equiv \frac{n_{\mu t}}{n_{\mu t}+n_{\mu}} (\text{or } \alpha_{\mu d}) \text{ and } \alpha_{t^+} \equiv \frac{n_{d^+}}{n_0} (\text{or } \alpha_{t^+}) \qquad (4)$$

to be sufficiently large, where n is the number density and n_0 is the number density of dt particles in the pellet.

Now, the ionisation potentials for the μt (or μd) and et (or ed) atoms are, respectively, 2.7 keV and 13.5 eV. Applying Saha's equation on equilibrium constants to the reactions (3) we obtain $\alpha_{\mu t}$ (or $\alpha_{\mu d}$) and α_{t^+} (or α_{d^+}) (Fig. 2). The reaction $\mu + t^+ \rightleftharpoons \mu t$ (or $\mu + d^+ \rightleftharpoons \mu d$) is in equilibrium because its reaction time is $\sim 10^{-12}$ s (the case of low energy ionisation loss of ref. 1) at solid density and proportionately smaller at higher density. Thus, compared with a confinement time of 1 ns the dissociation/recombination time is small. The reaction $e + t^+ \rightleftharpoons et$ (or $e + d^+ \rightleftharpoons ed$) has a much shorter time than 10^{-12} s at solid density since its cross section is obviously larger than for the reaction $\mu + t^+ \rightleftharpoons et$.

Both $\alpha_{\mu t}$ (or $\alpha_{\mu t}$) and α_{t^+} (or α_{d^+}) are sufficiently large in the density and temperature range envisaged, for catalytic fusion to take place almost up to ignition point (see Fig. 2). When the catalytic reactions are nearly complete the ignition temperature would be reached and the main burn will take place by means of ordinary fusion reactions.

Output

For an idea of the overall energy output over input ratio we consider a few cases. The parametric relations used are (where number densities are for one pulse)

$$E_t = x_b N T_i / R_b = n_\mu T_t R_c x_r \qquad (5)$$

Fig. 2 Ionisation fractions. $\alpha_{\mu t}$ ———, (possible implosion path ——→——); α_{t^+} — — — —, (possible implosion path — — → — —).

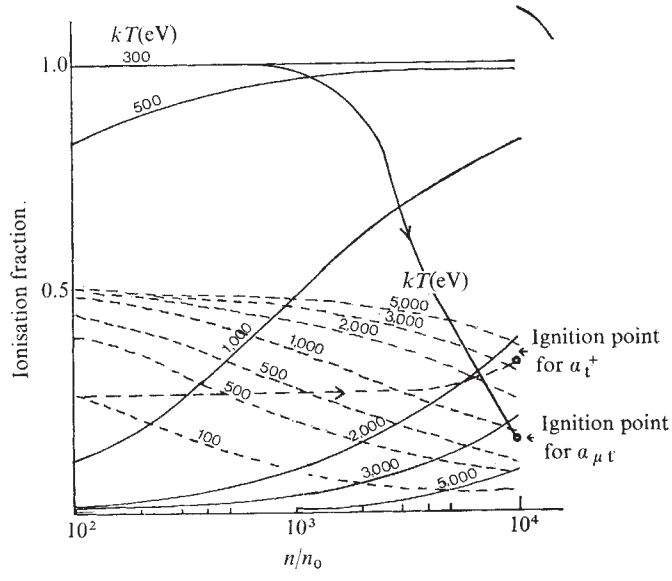

Table 1 Limits to fusion parameters

Parameter	Minimum	Maximum	Remarks
N	2×10^{19}	2×10^{22}	Lower limit comes from a pellet of 0.5 mm radius, possibly the smallest pellet consistent with focusing of muon beam
x_b	0.01	0.1	Lower limit is the 1% burn fraction as calculated by Brueckner[8] for a 0.5-mm pellet in laser driven fusion
R_b	200	2,000	Lower limit from laser driven fusion[8]
T_i	← 3.5 KeV →		T_i = 3.5 keV is the result of large R_b as given by Lawson[10]
T_r	3.5 MeV	17.6 MeV	Lower limit is the result of α particles stopped in core zone. Upper limit is the result of both α and n particles stopped in core zone
R_c	1	100	Upper limit has a firm theoretical basis[4,6]
x_r	0.1	1.0	Upper limit implies that all neutrons and α-particles are stopped in ignition zone (possible for 10^4 compression)
x_μ	10^{-4}	10^{-2}(?)	Lower limit has a firm experimental basis[9]
e_μ	10^9 eV	10^{10} eV	Upper limit is a reasonable estimate in 1957[4]

where E_t is the thermal energy required as well as the energy available for ignition. N = number of d and t particles in the pellet; x_b = fraction of these particles burned; R_b = burn–ignition particle ratio; T_i = ignition temperature in eV; n_μ = number of muons injected into the pellet and assumed also to be the number of muons collimated into a beam; T_r = energy of reaction products; R_c = catalytic chain ratio; x_r = fraction of reaction products stopped in the ignition zone.

If N_μ is the total number of muons produced from the electrical mains, e_μ is the mains energy (eV) required to produce one muon and X_μ is the fraction of muons collimated into a beam then the total input energy E_M is

$$E_M = e_\mu N_\mu = \frac{e_\mu n_\mu}{x_\mu} = \frac{e_\mu x_b N T_i}{x_\mu x_r R_b R_c T_r} \tag{6}$$

The fusion energy produced per pulse is

$$E_F \simeq e_f x_b N \tag{7}$$

Table 2 Possible parameter combination

Cases	1*	2†	1a*	2a†
N	2×10^{19}	2×10^{22}	↑	↑
x_b	0.01	0.1		
R_b	200	2,000	as in Case 1	as in Case 2
T_i(keV)	3.5	3.5		
T_r(MeV)	3.5	17.6		
R_c	100	100		
x_r	1.0	1.0		
x_μ	10^{-2}	10^{-2}	10^{-4}	10^{-4}
e_μ	10^9	10^9	10^{10}	10^{10}
E_t(eV)	3.5×10^{18}	3.5×10^{21}	3.5×10^{18}	3.5×10^{21}
n_μ	10^{10}	2×10^{12}	10^{10}	2×10^{12}
N_μ	10^{12}	2×10^{14}	10^{14}	2×10^{16}
E_M(eV)	10^{21}	2×10^{23}	10^{24}	2×10^{26}
E_F(eV)	3.5×10^{24}	3.5×10^{28}	3.5×10^{24}	3.5×10^{28}
E_F/E_M	3.5×10^3	1.75×10^5	3.5	1.75×10^2

* Cases 1 and 1a are for the minimum size pellet.
† Cases 2 and 2a are for the maximum size pellet.
Cases 1a, 2a have worse muon collimation (x_μ) and input energy requirement (e_μ) characteristics than cases 1, 2 respectively.
E_t, n_μ, N_μ, E_M, E_F and E_F/E_M are the results of assumptions made in the values for N, x_b, R_b, T_i, T_r, R_c, x_r, x_μ and e_μ.

where e_f = 17.6 MeV is the energy produced in a deuterium–tritium fusion reaction, and where the contributions from the catalytic reactions are neglected since the burn–ignition ratio is normally large. The overall energy output over input ratio is thus

$$E_F/E_M = \frac{e_f}{e_\mu}\frac{x_\mu x_r R_b R_c T_r}{T_i} \tag{8}$$

The probable upper and lower limits of the parameters in equation (8) are given in Table 1. Four possible combinations of these parameters are given in Table 2. These are for two pellet sizes: a small pellet of ~ 1 mm diameter, as used in laser fusion studies, and a larger one of ~ 1 cm diameter. Though it appears from equation (8) that E_F/E_M is independent of pellet size (that is, N) in practice the parameters x_μ, x_r, R_b, R_c and T_r are probably dependent on it; x_b, R_b and T_r probably more so than the others, and are chosen accordingly in Table 2. Thus T_r is chosen to be 17.6 MeV for the larger pellet since it is thought probable that both neutrons and α particles are stopped at the core, whereas only the α particles are stopped in the case of the smaller pellet. Similarly, R_b may be an order of magnitude larger for the larger pellet if the compressed core is an order of magnitude larger than the core of the smaller pellet.

In the four case studies of Table 2, E_F/E_M varies widely from 3.5 to $>10^5$ and detailed computer simulation of the implosion hydrodynamics is necessary to clarify this point, though one obvious conclusion is that an improvement in the muon collimation (that is, in x_μ) would result in a positive gain to E_F/E_M.

At this stage it may be pointed out that, following the examples of laser fusion computer studies, most of the energy absorbed by the pellet will go into the compression of the core and only a little more is needed for ignition. Thus it may be objected that there is no need for the heating contribution of muon catalysed reactions. This, however, is only true for a small pellet under conditions optimum to laser induced implosions. For a larger pellet with a large core region and with the compression ratio at the edge of the core $\ll 10^4$ the confinement time criterion may still be satisfied while substantial core heating may be necessary for ignition. This is where the muon catalysed reactions would prove useful. Also unlike laser-driven fusion, electrons which are degenerate at high densities, need not be heated at all (though they will be heated as a matter of course by the pre-pulse) and this will reduce the thermal energy requirement.

Muon production

At present muons are most efficiently produced from the decay of pions, which are themselves products of the collisions of energetic protons with a suitable target. At SINR, and elsewhere, muon 'factories' have recently come into operation, capable of producing muon pulses of $>10^7$ particles from 10^{11} protons, that is, $x_\mu \simeq 10^{-4}$. The reason for such poor collimation is that as pions decay the muons are emitted isotropically. Another problem is to obtain a large pulse in a short time. Here a storage ring with a fast outlet shutter could be employed. The particles stored can be either protons or muons. The former have an infinite lifetime so that the injection current (into the ring) need only be large enough to satisfy the rate of explosions required by the reactor. On the other hand, if the muon collimation is poor, a far larger number of particles has to be stored in the ring if the particles are protons than if they are muons. Muons, when initially produced, have energies too high for the muons to be stopped by a small pellet. They will, therefore, have to be decelerated by some means, perhaps by the emission of cyclotron radiation in the storage ring. If muons are stored in a ring the injection current has a lower limit determined by the mean lifetime and the output current pulse. A muon storage ring has recently been commissioned at CERN.

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Evidence for the Azores mantle plume from strontium isotope geochemistry of the Central North Atlantic

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Strontium isotope variations in basalts from the Mid-Atlantic Ridge and the Azores Islands support the Azores mantle plume model. The similarity of isotope ratios in Mid-Atlantic Ridge tholeiites from the Azores platform and Azores Islands alkali basalts implies that these different magma types are derived from the same mantle source but under different conditions of origin.

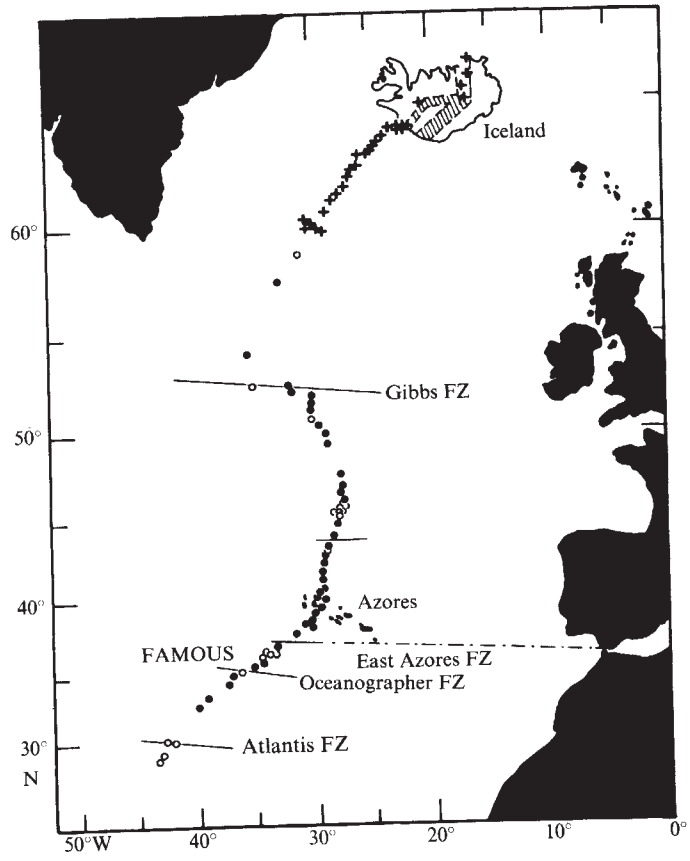
THE two dominant modes of igneous activity in ocean basins, oceanic island and mid-ocean ridge, produce magmas of distinctly differing geochemistry[1-3]. Mid-ocean ridge basalts are predominantly tholeiitic and have very low large ion-lithophile (LIL) element abundances and low (large ion)/(small ion) abundance ratios of LIL elements such as Rb/K, Ba/Sr, and La/Sm. On the other hand, oceanic island lavas may be of either the tholeiitic or alkaline type, both of which have distinctly higher LIL element concentrations, higher LIL element ratios, and generally higher abundances of radiogenic Sr and Pb[2]. As both MOR and oceanic island volcanics are derived from the mantle, this contrast implies significant mantle heterogeneity and differences in mantle evolution.

The Azores archipelago consists of nine volcanic islands which emerge from a shallow submarine plateau straddling the Mid-Atlantic Ridge (MAR) and the triple junction of the North American, Eurasian, and African plates[4] at approximately 40°N. Because of the closely related setting of the Azores Islands, the submerged plateau, and the transecting MAR axis, the region is excellent for studying the geochemical contrasts and spatial relations of mid-ocean ridge and oceanic island basalts.

A previous study by Schilling[5] showed that tholeiitic basalts erupted along the MAR transect of the Azores platform have patterns enriched in light rare earths which grade regularly southwards along the ridge axis to patterns depleted in light rare earths, typical of mid-ocean ridge basalts, at 33°N. Because these trace element variations could not reasonably be accounted for by different degrees of partial melting or fractional crystallisation, Schilling interpreted this gradient as resulting from a mantle plume[7] enriched in LIL elements, rising beneath the Azores and mixing southwards along the ridge with the source of normal ridge basalts 'depleted' in LIL elements in the low velocity layer. $(La/Sm)_{c.f.}$ ratios were also found to decrease northwards from 45°N to the Gibbs Fracture Zone[6].

Since trace element ratios can be affected to some extent by processes such as fractional crystallisation and partial melting, further documentation of the geochemical variation observed by Schilling was desirable. In particular, whereas the La/Sm ratio in tholeiites may be taken as representative of the source (if they are produced by at least 20% melting of a peridotitic mantle), this is not the case for magmas which may be produced by smaller degrees of melting, such as alkali basalts[8]. For this reason, Schilling did not study the relationship between the alkaline Azores island lavas and lavas from the MAR transect of the Azores platform. Sr-isotope ratios, because of their relative insensitivity to magmatic processes, are useful in examining these relationships.

Fig. 1 Map of the study area. ●, RV Trident dredge stations; ○, other dredge stations; ×, locations of dredge stations for which $^{87}Sr/^{86}Sr$ was reported by Hart *et al.*[12].



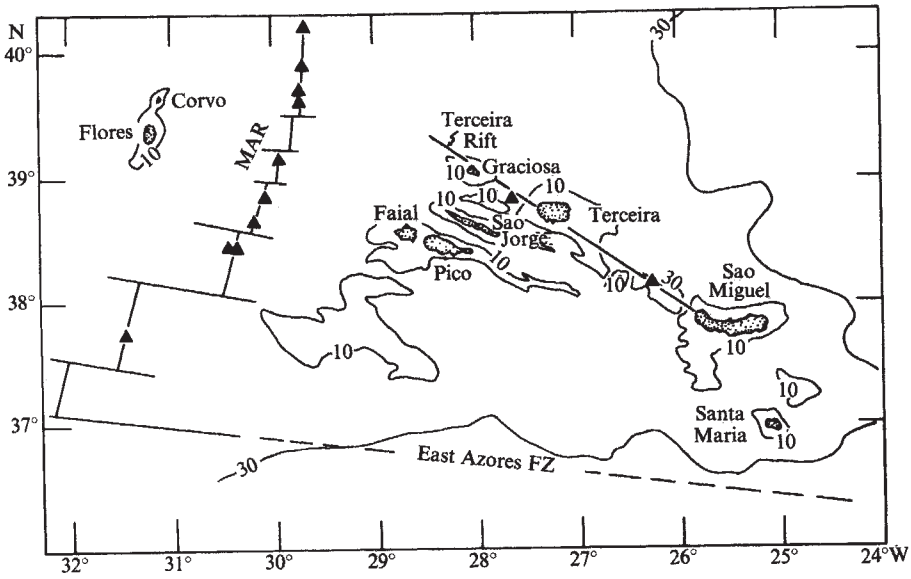


Fig. 2 Map of the Azores region. ▲, Dredge stations. 1,000- and 3,000-m contours are shown.

We now report the results of a Sr-isotope study of lavas erupted along the MAR from 29°N to 60°N, on the Azores Islands, and in the Terceira trough. The analyses were carried out on powder splits of the same MAR dredge samples on which rare earths had been determined previously by Schilling[5] and Schilling *et al.*[6]. The present study further documents geochemical variations in the region and provides a test of the Azores mantle plume hypothesis and the mixing model[5] based on rare earth elements. We also examine the relationship of alkali lavas erupted on the Azores Islands to the tholeiitic basalts erupted on the nearby MAR.

Seventy-nine samples from the rift valley of the Mid-Atlantic Ridge and the Terceira trough were analysed. Locations of the dredge stations are shown in Figs 1 and 2. Samples were dredged by RV Trident of the University of Rhode Island during cruises TR89, TR100, TR119, TR122, TR123, and TR154. Additional samples from other dredge stations were provided by other investigators. Thirty-five samples from the Azores Islands were also analysed—their locations are shown in Fig. 3. Practically all the samples from the MAR are tholeiitic basalts, but rare transitional and one mildly alkaline basalt occur near 35°N (just north of the Oceanographer Fracture Zone) and at 45°N[5,9,10]. Lavas from the Azores Islands are alkalic.

$^{87}Sr/^{86}Sr$ ratios were measured using the 6-inch mass spectrometer at the Department of Terrestrial Magnetism, Carnegie Institution of Washington. The analytical technique has been described elsewhere[11,12]. Our data from Terceira Island and the southern Reykjanes Ridge agree with values reported by O'Nions and Pankhurst[13], after correction for an apparent interlaboratory bias of 0.014%. Because of the low Rb/Sr ratios and the relatively young age of the basalts, isotope ratios in these samples can be considered 'initial'. The comenditic lavas have high Rb/Sr ratios, however, and the $^{87}Sr/^{86}Sr$ ratios reported for these samples cannot be strictly considered 'initial'.

Geochemical variation along the ridge

The $^{87}Sr/^{86}Sr$ profile of basalts erupted along the ridge is shown in Fig. 4. Two distinct maxima are apparent, one at 44–45°N, the other over the Azores platform (39°N). The maximum over the Azores platform seems slightly higher (about 0.70345) than that at 45°N (about 0.70340). Transition zones exist north of 45°N and south of 39°N with the $^{87}Sr/^{86}Sr$ ratios decreasing to typical ridge values. Ten analysed samples from the FAMOUS region, situated in the geochemical transition zone south of the Azores, fall in a narrow range of intermediate values in spite of a large variation in bulk chemistry[14]. The profile of $(La/Sm)_{e.f.}$ (ref. 5) along this section of the ridge, as well as those of alkali and alkaline earth trace elements and ratios such as Rb/K, Ba/K, and Rb/Sr are similar to the strontium isotope profile[15]. All these different sets of data suggest that the areas south of 33°N and between 48°N and 60°N are 'normal ridge segments'.

$^{87}Sr/^{86}Sr$ ratios for samples from three dredge stations in the 35°N–Oceanographer Fracture Zone area are anomalously high. Although these samples show some evidence of secondary alteration, their K/Cs ratios (a good indicator of alteration[16]) are relatively high and suggest that these high $^{87}Sr/^{86}Sr$ ratios are not due to secondary alteration. Nor do the anomalously high strontium isotope ratios in this area represent a difference between rift valley and fracture zone volcanism. One dredge station is located within the MAR rift valley just north of the fracture zone. The second is located south of the fracture zone, though not necessarily in the rift (which could not be identified because of the complex topography of the area). The third is located within the fracture zone[17].

$^{87}Sr/^{86}Sr$ values for station TR123 4D (33°N) are also anomalously high. These samples are quite unusual in composition, having extremely low LIL element concentrations and low ratios such as La/Sm and Rb/K. Petrologically, they are Al-rich picrites similar to rocks from DSDP sites 3–14 and 3–18[8,18] and contain Al-rich spinels[19].

Variation patterns in the Azores

The samples from the Azores Islands vary from alkali basalts to highly differentiated comendites. Submarine basalts from the Terceira trough are tholeiitic[20]. Initial $^{87}Sr/^{86}Sr$ ratios from six of the nine islands fall in the narrow range of 0.70332 to 0.70354, and thus are identical to $^{87}Sr/^{86}Sr$ ratios of basalts from the MAR transect of the platform (Fig. 5). Figure 5 shows also that there is no systematic change in $^{87}Sr/^{86}Sr$ ratios longitudinally away from the ridge. Both island lavas and tholeiites from the ridge transect of the platform have strontium isotope ratios which are distinct from normal ridge segments.

Flores and Corvo are the only two islands west of the MAR. $^{87}Sr/^{86}Sr$ values for these two islands range from 0.70332 to 0.70352, with no apparent isotopic differences between the two islands. $^{87}Sr/^{86}Sr$ ratios from Faial and Pico range from 0.70347 to 0.70394, with values from Faial tending to be somewhat higher than those from Pico. Sao Jorge has $^{87}Sr/^{86}Sr$ ratios which range from 0.70332 to 0.70354.

Graciosa, Terceira and Sao Miguel lie along the Terceira trough, which may be a secondary spreading centre[4]. Lavas from Graciosa and Terceira, and submarine tholeiites from the trough have $^{87}Sr/^{86}Sr$ ratios in the range 0.70336 to 0.70354, with the exception of two comendites from Graciosa and

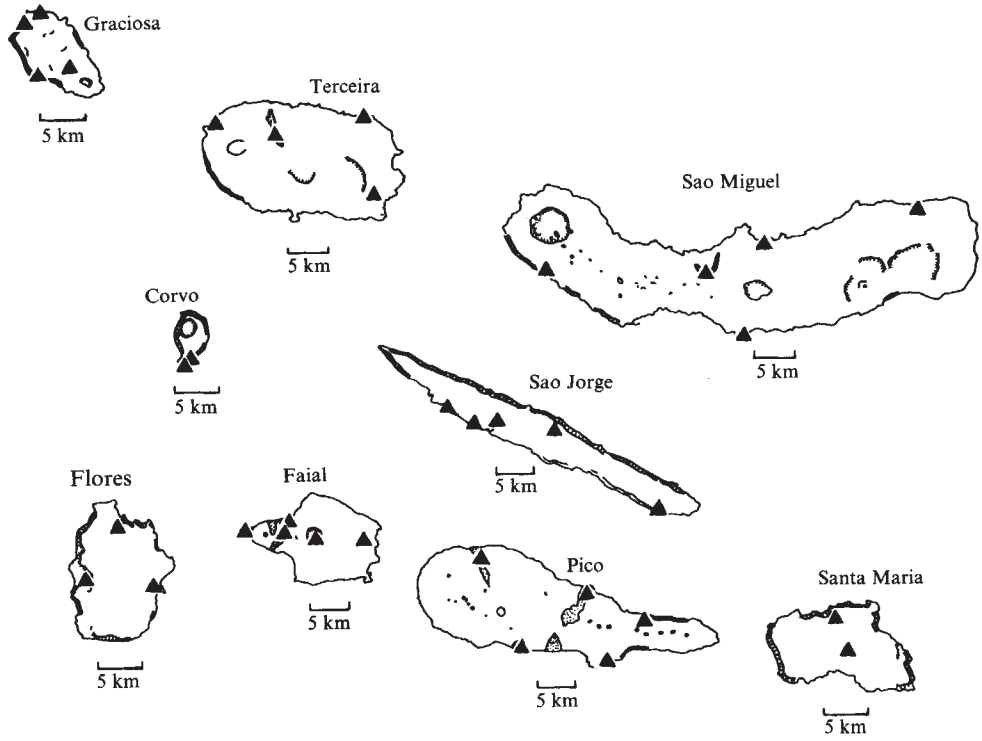


Fig. 3 Sample location map for the Azores Islands. ▲, Sample locations; dotted areas, historical lava flows.

Terceira. The high Rb/Sr ratios, 8 and 10, in the two comendites suggest that their high $^{87}Sr/^{86}Sr$ ratios may be the result of ^{87}Rb decay since differentiation of the magma. If the initial ratios of these two samples were near 0.70350, then the apparent ages are 2.5 Myr for the Graciosa sample and 0.75 Myr for the Terceira sample. Field relationships suggest that these ages may be reasonable.

Sao Miguel, which is apparently located at the intersection of the Terceira, Sao Jorge and Faial–Pico tectonic lineaments[21], is geochemically quite distinct from the other islands. One basalt has an $^{87}Sr/^{86}Sr$ ratio of 0.70337, whereas four other values range from 0.70434 to 0.70525. Samples from Santa Maria have $^{87}Sr/^{86}Sr$ ratios of 0.70336 and 0.70352.

Thus Faial, Pico and, particularly, Sao Miguel are the only islands where initial $^{87}Sr/^{86}Sr$ ratios are markedly dissimilar to those of the adjacent MAR. All these samples are fresh and the high $^{87}Sr/^{86}Sr$ ratios cannot be explained by post-eruptional alteration.

Comparison with Iceland

The maximal $^{87}Sr/^{86}Sr$ and $(La/Sm)_{c.r.}$ values for the ridge transect of the Azores platform (0.70345 and 2.8 (ref. 5) respectively) are considerably higher than values for the ridge transect across Iceland (0.70305 (ref. 12) and 1.3 (ref. 22) respectively). This is readily apparent for $^{87}Sr/^{86}Sr$ in Fig. 4. Additionally, although the Iceland–Reykjanes Ridge transition zone between the 'undepleted' Icelandic basalts and the 'depleted' ridge basalts extends only some 400 km, the transition zone between the Azores platform and the 'normal' ridge to the south extends some 1,000 km. Thus the geochemical anomaly beneath the Azores is both richer in LIL elements and larger than the anomaly beneath Iceland.

Single or multiple mantle source?

The large scale geochemical gradients along the Reykjanes Ridge reported by Schilling[22], Hart *et al.*[12] and Sun *et al.*[23] have been explained by these authors in terms of mixing of two mantle sources. This model was challenged by O'Hara[24,25] who suggested that the LIL element variations along the Reykjanes Ridge could be produced by fractional crystallisation. O'Hara[24] dismissed the isotope evidence, stating that the "isotope ratios had changed during the manifest fractional crystallisation." As O'Hara has not yet proposed a mechanism capable of producing such changes and as we know of no evidence to support this contention, we consider it improbable that Sr isotope ratios can be affected by fractional crystallisation.

Other alternatives to the multiple-source interpretation of the data from the Reykjanes Ridge have been based on possible

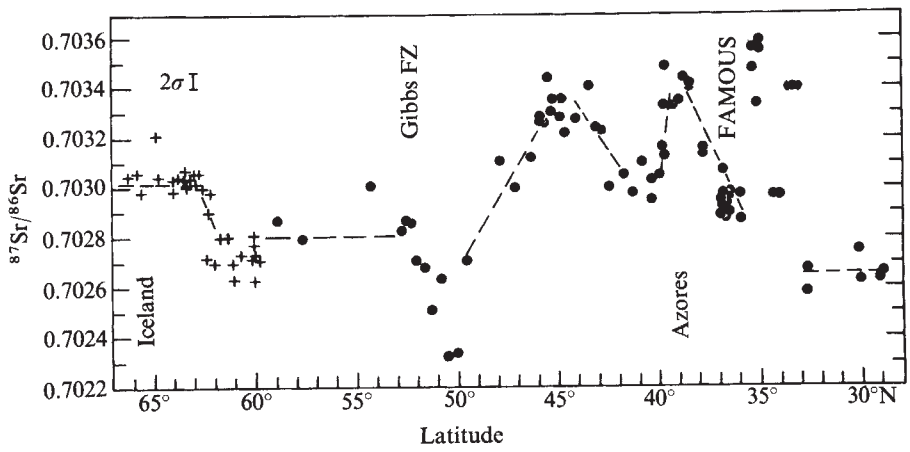


Fig. 4 Variation of $^{87}Sr/^{86}Sr$ with latitude along the Mid-Atlantic Ridge. Precision is indicated by the error bar (upper left) and is ±0.00006 (±2 s.d.). Measured values for the E and A NBS-SRM 987 standards were 0.70792 ±2 (six analyses) and 0.71017 ±5 (six analyses) (2 s.d. of the mean). All values were normalised to 86/88=0.1194 and are reported relative to a value of 0.70800 for the E and A standard. Data previously reported by Hart *et al.*[12] are shown for comparison (+). The dashed line is interpretive.

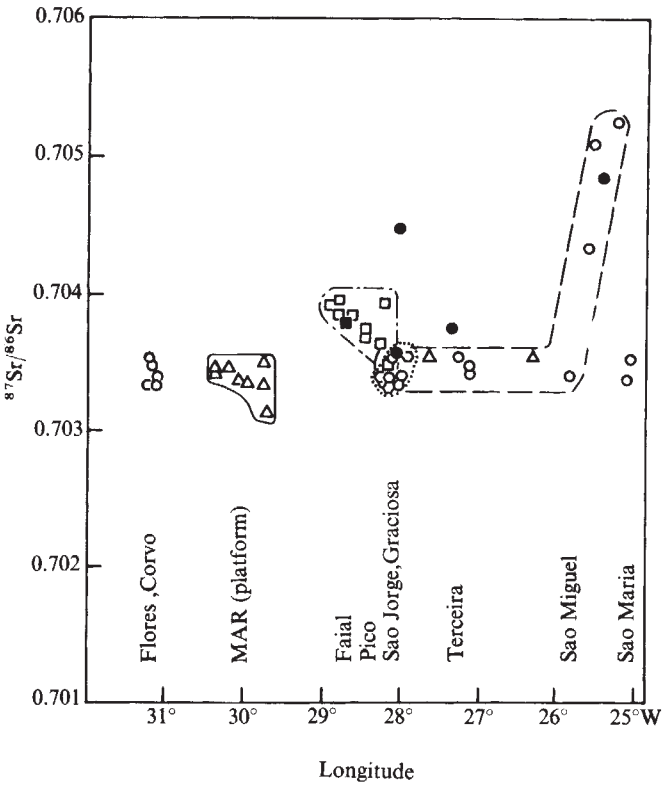


Fig. 5 Variation of $^{87}Sr/^{86}Sr$ with longitude across the Azores plateau. △, Dredge stations; □, Faial and Pico; hexagons, Sao Jorge; ○, Flores, Corvo, Santa Maria, Graciosa, Terceira and Sao Miguel. Open symbols are basalts and trachybasalts, filled symbols are trachytes. Lines enclose samples from the principal tectonic features of the Azores[21]: - - -, Terceira Rift; · · ·, Sao Jorge; · - · - · -, Faial–Pico; ——, MAR.

disequilibrium melting of a phlogopite-bearing mantle[13,26-28]. Disequilibrium melting models such as these are not supported by our results in the Azores region. The correlation of $^{87}Sr/^{86}Sr$ with elevation along the Reykjanes Ridge, on which Flower *et al.*[26] based their model, is poor or nonexistent in the present results. The minimum depth of the rift valley of the MAR transect of the Azores platform is about 1,100 m, yet the strontium isotope ratios of these samples are considerably higher than those of subaereal Icelandic basalts. Sigvaldason's model[27], suggesting that the Iceland mantle plume feeds volcanism in all of the North Atlantic, cannot explain the high $^{87}Sr/^{86}Sr$ values at 45°N and the Azores platform, or the associated gradients.

O'Nions and Pankhurst[13] have developed a disequilibrium melting model for a phlogopite-bearing mantle. A corollary of this model requires that the $^{87}Sr/^{86}Sr$ ratio of the melt should decrease with increasing degree of melting. If alkali basalts are produced by smaller degrees of melting than tholeiites, as suggested by experimental data[29], alkali basalts should have higher strontium isotope ratios than tholeiites derived from the same source. Contrary to this prediction, however, most alkali lavas from the Azores Islands have $^{87}Sr/^{86}Sr$ ratios nearly identical to those of tholeiites from the MAR transect of the Azores plateau. In addition, basalts of tholeiitic composition dredged from the Terceira trough have isotope ratios within the range of alkali basalts from the nearby islands of Terceira and Graciosa.

A number of other serious objections to the disequilibrium melting hypothesis have been raised by Hofmann and Hart[30]. In view of these objections and the above discussion, the disequilibrium melting model seems inapplicable to our results. We conclude that the observed variation in lavas from the MAR and the Azores Islands does indeed reflect geochemical variation in the mantle.

Any model which attempts to account for the isotopic variation along the ridge must also account for (1) the increases in trace elements and trace element ratios towards the Azores[5,15]; (2) petrological and petrochemical variation along the ridge (ref. 19 and J.-G. S. and W. M. W., to be published); (3) the broadening and shoaling of the MAR in this region; and (4) the thicker crust present beneath the Azores platform by comparison with typical oceanic crust[31].

At present, the model we prefer to account for these observations is that of a mantle plume[5,7] rising beneath the Azores (with a possible separate plume at 45°N) and mixing with the source (depleted in LIL elements) of normal ridge basalts. Reasons for the choice of this model over other models such as a vertically or horizontally heterogeneous static mantle have been discussed elsewhere[3,5,12,15,22]. We interpret the contrast of the alkalic nature of the Azores Islands lavas with the tholeiitic character of basalts from the MAR transect of the platform as being due primarily to differences in conditions of magma generation and evolution, such as depth and extent of melting and subsequent crystal fractionation, rather than differences in mantle source chemistry[29]. These different conditions of magma formation generally produce some enrichment in LIL elements in the melts but do not produce isotopic differences. Higher Sr isotope ratios on Faial, Pico and, particularly, Sao Miquel imply considerable local mantle heterogeneity in this region, however.

Strontium isotope ratios do not decrease longitudinally across the platform (a distance of over 500 km), whereas they begin decreasing along the MAR within 100 to 200 km from the centre of the platform. This asymmetry may result from the fact that although there is mixing of the plume and depleted low velocity layer sources along the ridge, only the plume contributes material to island volcanism. Our analyses of island lavas include both historical flows and flows which are from some of the oldest volcanic sequences of the archipelago (Santa Maria and north-eastern Sao Miguel[32]) and reveal no particular systematic change with time. Thus the plume must have been present beneath the Azores for at least the past 4–6 Myr, and probably longer, judging from rare earth data in DSDP Leg 37 basalts[33].

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An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation

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Microsomes prepared from rabbit or pig aortas transformed endoperoxides (PGG_2 or PGH_2) to an unstable substance (PGX) that inhibited human platelet aggregation. PGX was 30 times more potent in this respect than prostaglandin E_1. PGX contracted some gastrointestinal smooth muscle and relaxed certain isolated blood vessels. Prostaglandin endoperoxides cause platelet aggregation possibly through the generation by platelets of thromboxane A_2. Generation of PGX by vessel walls could be the biochemical mechanism underlying their unique ability to resist platelet adhesion. A balance between formation of anti- and pro-aggregatory substances by enzymes could also contribute to the maintenance of the integrity of vascular endothelium and explain the mechanism of formation of intra-arterial thrombi in certain physiopathological conditions.

THE prostaglandin (PG) endoperoxides (PGG_2 and PGH_2) are generated from arachidonic acid by the membrane-bound cyclo-oxygenase enzyme[1–3], and subsequently transformed to $PGF_{2\alpha}$, E_2 or D_2 (or to their 15-hydroperoxy or 15-keto derivatives) or to 12-L-hydroxy-5,8,10-heptadecatrienoic acid (HHT) and malondialdehyde[1,3,4]. Recently a novel transformation of PGG_2 and H_2 to a non-prostaglandin compound "thromboxane A_2" (TXA_2) has been reported[3,5], and a microsomal thromboxane synthetase system in blood platelets has been identified and characterised[6,7]. Most of the activity associated with "rabbit aorta contracting substance" or RCS[8,9] is now thought to be due to TXA_2 (ref. 10). TXA_2 shares with prostaglandin endoperoxides two important biological properties; they both contract strips of rabbit aorta[6,7,10,11], and cause platelet aggregation *in vitro*[5,12].

We have discovered that blood vessel microsomes contain an enzyme that transforms PG endoperoxides to an unstable principle which relaxes some blood vessels and prevents platelet aggregation.

Preparation of enzyme and bioassay

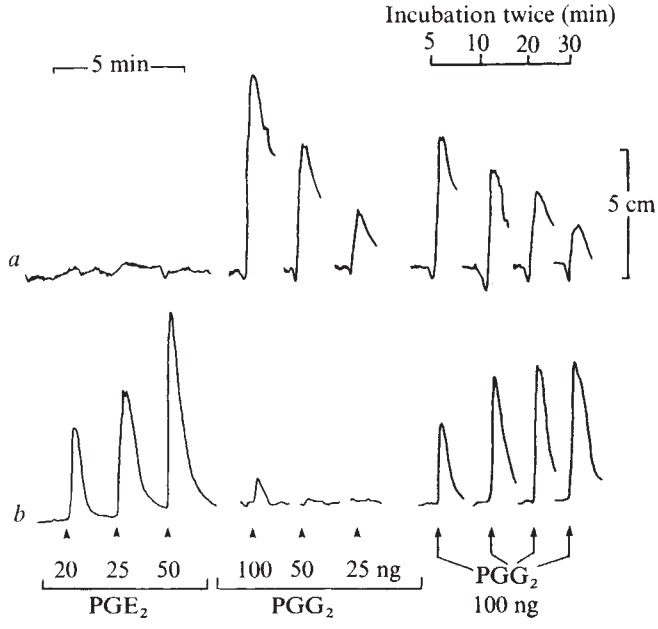
Pig or rabbit aortas (one experiment) were stripped of adventitia, snap-frozen in liquid nitrogen, crushed into a fine powder, resuspended (1:4, w/v) in 0.05 M Tris buffer (*p*H 7.5) and homogenised at high speed in a Polytron homogeniser. The homogenate was centrifuged at 1,000*g* for 15 min and the resulting supernatant centrifuged again at 10,000*g* for 5 min. The 10,000*g* pellet was also discarded and after further centrifugation at 100,000*g* for 60 min was resuspended in deionised water and lyophilised. Yield averaged 150 mg powder (51% protein)[13] per 100 g aortic tissue. Electron microscopic examination showed that this fraction was composed mainly of microsomes although there was a small contamination with mitochondria.

Activity of PGs and products of the PG endoperoxides was assayed using the cascade superfusion technique[14]. Tissues were selected from spirally cut strips of rabbit aorta, pulmonary artery, mesenteric artery, coeliac artery and vena cava, as well as rat stomach strip, rat colon, chick rectum, guinea pig ileum and guinea pig tracheal chain. These were superfused in cascade with Krebs' solution (at 37 °C) at 10 ml min^{-1}, containing a mixture of antagonists[15] and indomethacin (1 μg ml^{-1}). Changes in length of the tissues were detected using Harvard smooth muscle transducers and recorded at an overall magnification of 1–4 times on a Watanabe six-channel pen recorder.

The most useful selection of assay tissues was rabbit aorta, rat colon and rat stomach. Rabbit aorta is contracted by PG endoperoxides and TXA_2 but not by PGE_2, $PGF_{2\alpha}$ or PGD_2. Rat colon is contracted by PGE_2 and $PGF_{2\alpha}$ but not by PGH_2, PGG_2 or TXA_2 (refs 9 and 16). Rat stomach strip is contracted to different degrees by all of the above substances.

Aortic microsomes (10–1,000 μg) were incubated with substrate in 100 μl 0.05 M Tris buffer (*p*H 7.5). No cofactors were added. The following substances were tested as substrates: arachidonic acid (1–10 μg ml^{-1}), prostaglandins E_2, $F_{2\alpha}$, D_2, G_2 and H_2 (0.1–1.0 μg ml^{-1}). Reaction sets were incubated

Fig. 1 Bioassay using rabbit aortic strip (*a*) and rat colon (*b*). PGE_2 contracts rat colon whereas PGG_2 contracts rabbit aorta. PGG_2 (0.5 μg) was incubated in 0.5 ml Tris buffer (0.5 M, *p*H 7.5) at 22 °C, and 100-μl aliquots were tested on the tissues at 5, 10, 20 and 30 min. The spontaneous disappearance of PGG_2 (decrease in rabbit aorta contraction) was associated with an appearance of PGE- and F-like activity (increase in rat colon contraction).



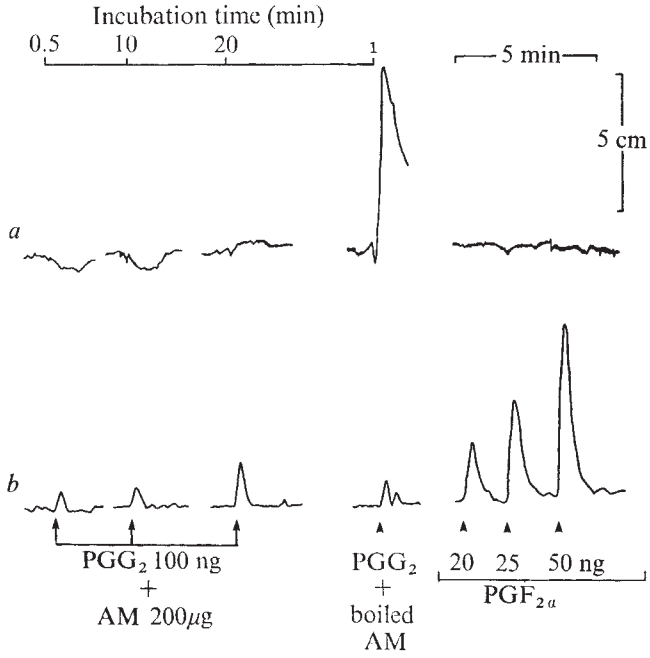


Fig. 2 Effect of aortic microsomes (AM) on PGG_2. PGG_2 (0.5 μg) was incubated with AM (1 mg) in 0.5 ml of Tris buffer (0.05 M, *p*H 7.5) at 22 °C and 100-μl aliquots were tested for activity on the tissues at 0.5, 10 and 20 min. *a*, Rabbit aortic strip; *b*, rat colon. In the presence of AM the contractile activity of PGG_2 on rabbit aortic strip disappeared within 0.5 min but no PGE- or F-like activity was formed even when incubated for 20 min. Boiled AM (200 μg) incubated with PGG_2 (100 ng) in 100 μl Tris buffer at 22 °C for 1 min did not affect activity of PGG_2 on rabbit aorta. The last section shows the selective effect of $PGF_{2\alpha}$ on the rat colon.

for 0.2–30 min at room temperature (20–22 °C). Aliquots of the incubation mixture were tested directly on the assay tissues to detect changes in activity induced by the enzyme. PGG_2 and PGH_2 are unstable in aqueous solution, with a half life of 7–8 min at 22 °C (ref. 7). As they decompose (Fig. 1), so the activity on rabbit aorta decreases and PGE- and F-like activity on rat colon increases.

When incubated with aortic microsomes (3–4 mg ml^{-1}) for 0.25–0.5 min at 22 °C, the contractor activity of PGG_2 or PGH_2 (1 μg ml^{-1}) disappeared, without any increase in PGE- or F-like activity on the rat colon (Fig. 2). There was also no increase in malondialdehyde formation, as estimated by the thiobarbituric acid method[17]. The endoperoxides were transformed, however, into a substance with a different spectrum of biological activity. For convenience, we shall refer to this unknown substance as PGX.

PGE_2, $PGF_{2\alpha}$, PGD_2 (0.5–5 μg ml^{-1}) or arachidonic acid (1–25 μg ml^{-1}) were incubated with aortic microsomes for up to 30 min at 22 °C; since there was no detectable change in biological activity, it was concluded that these substances were not substrates for the enzyme which generates PGX.

The enzymic nature of the transformation by aortic microsomes of PGG_2 and PGH_2 to PGX was substantiated by the following observations: boiled microsomal preparations had no activity, and non-enzymic proteins (for example, bovine serum albumin) did not affect the spontaneous decay of PGH_2 during 1 min of incubation.

Effects of PGX

PGX (100 ng), whether extracted (see below) or not, did not contract strips of rabbit aorta, pulmonary artery or vena cava. PGX relaxed strips of rabbit mesenteric and coeliac arteries. PGX contracted rat stomach strip, chick rectum, guinea pig tracheal chain and guinea pig ileum, although its potency was less than that of PGH_2 or PGG_2. Rat colon was not contracted by endoperoxides or by PGX (100 ng), except after spontaneous decomposition of the prostaglandin endoperoxides (Fig. 1), when there was a 25–40% conversion to PGE- or F-like substances.

Aggregation of platelets in 1 ml fresh human platelet plasma (PRP) was monitored in a Born aggregometer[18]. Platelet aggregation was induced by arachidonic acid (100–600 μg or 0.3–1.8 mM). Aortic microsomes (5 mg) were incubated with 1 μg endoperoxide (PGG_2 or PGH_2) in 1 ml 0.05 Tris buffer (*p*H 7.5) for 2 min at 22 °C. Doses of PGX stated below assume complete conversion of the endoperoxide. An aliquot containing 0.25–10 ng PGX was added to the PRP 0.5–1 min before the aggregatory agent. In another set of experiments the products formed during incubation of aortic microsomes with PGG_2 or PGH_2 (incubation time 1–2 min at 22 °C) were rapidly extracted with ice-cold diethyl ether. After evaporation of the ether the residue was either dissolved in ice-cold Tris buffer and immediately used in platelet aggregation studies or was dissolved in anhydrous acetone and stored at −20 °C for future use. These extracts were added to PRP 1 min before the aggregatory agent.

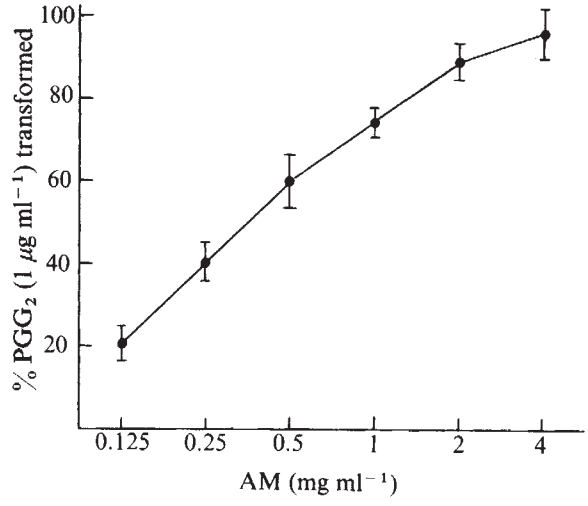
An immediate anti-aggregatory effect of the fresh reaction mixture of aortic microsomes with PGH_2 or PGG_2 was observed. The lowest anti-aggregatory concentration was obtained at concentrations of 0.5–5 ng PGX ml^{-1}. PGX was about 30 times more potent than PGE_1 and 5–20 times more potent than PGD_2 as an anti-aggregatory agent (Fig. 3). PGX was unstable and its anti-aggregatory activity disappeared after standing for 20 min at 22 °C or after boiling for 0.2 min. Aortic microsomes (50 μg ml^{-1}) alone could induce aggregation in some PRP. The products of spontaneous degradation of PGG_2 (100 ng ml^{-1}) had no anti-aggregatory activity.

Diethyl ether extracts of PGX also inhibited platelet aggregation induced by arachidonic acid. The effective anti-aggregatory concentrations of the extracted PGX ranged from 1–10 ng ml^{-1} (assuming complete extraction into ether and complete dissolution in Tris buffer).

The anti-aggregatory activity of the extracted PGX disappeared on boiling (15 s) or on standing at 22 °C for 20 min, but did not deteriorate when the buffered solution was kept on ice for 2 h or when the substance was dissolved in dry acetone and stored at −20 °C for several days.

PGX is different from the other products of PG endoperoxides so far described. Its biological properties on the isolated tissues, its instability and its potent anti-aggregatory activity distinguish it from PGE_2, $PGF_{2\alpha}$, TXA_2 or TXB_2. PGE_2,

Fig. 3 Transformation of PGG_2 activity by different concentrations of aortic microsomes, incubated with 100 ng of PGG_2 in 100 μl Tris buffer for 1 min at 22 °C, and bioassayed using rabbit aortic strip. The reduction in contractile activity of PGG_2 was calculated from the standard dose–response curve for PGG_2 as the percentage transformation of the endoperoxide. Each point on the graph represents the mean ±s.e. of five–seven experiments.



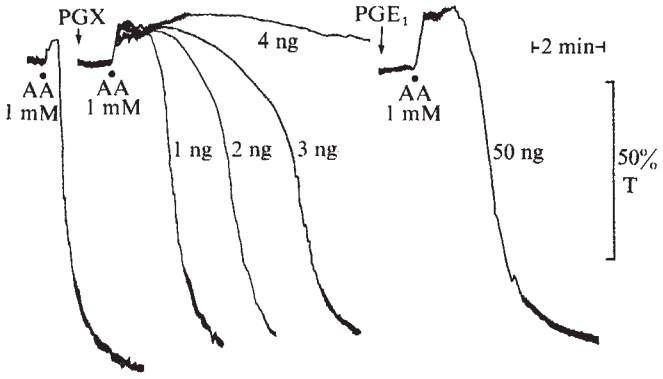


Fig. 4 The tracings show changes in light transmission through platelet-rich human plasma in a Born aggregometer. Comparison of anti-aggregatory potencies of PGX and PGE_1. PGX was obtained by incubation of 100 ng PGH_2 with 500 μg of aortic microsomes in 100 μl 0.05 M Tris buffer (*p*H 7.5) for 2 min at 22 °C and then stored on ice. PGX and PGE_1 (10 μl) were added to human platelet-rich plasma 1 min before arachidonic acid (AA 1 mM). In this experiment PGX was at least 25 times more potent as an anti-aggregatory agent than PGE_1. Doses of PGX (1–4 ng) or PGE_1 (50 ng) are shown at the sides of the tracings.

$PGF_{2\alpha}$ and PGD_2 were not substrates for aortic microsomes, so that 15-keto PGs or other products of PG catabolism could not be considered as PGX. PGX is also unlikely to be a 15-hydroperoxy PG, because, firstly, 15-OOH PGE_2 contracts rabbit aorta[2], and, secondly, the product(s) of the spontaneous decay of PGX when bioassayed did not behave like PGE_2, $PGF_{2\alpha}$ or PGD_2. Lack of increased malondialdehyde formation from PGG_2 and PGH_2 excluded their enzymic transformation by aortic microsomes to HHT.

The presence of prostaglandin D_2 isomerase in homogenates of several tissues has been described[2]. Prostaglandin D_2 is a potent inhibitor of platelet aggregation[19]. PGX is, however, not PGD_2 because PGX is unstable and is a more potent anti-aggregatory agent than PGD_2. Besides, PGD_2 isomerase is present in the 100,000*g* supernatant[2], a fraction that in our experiments did not produce PGX from endoperoxides. Moreover, PGD_2 isomerase needs glutathione as a cofactor[2] and our incubations were carried out in the absence of cofactors.

The prostaglandin endoperoxides (PGG_2 and PGH_2) aggregate platelets[10,12] and this activity is probably mediated, wholly or partly, by the enzymic conversion of endoperoxides by platelet microsomes to the less stable TXA_2 (refs 6 and 7). Blood vessel microsomes transform endoperoxides to another unstable principle (PGX) which, contrary to TXA_2, has potent anti-aggregatory properties and relaxed, rather than contracted, vascular strips. Thus a balance between the amounts of TXA_2 formed by platelets and PGX formed by vessels might be critical for thrombus formation. Indeed, in the light of the discovery of this anti-thrombotic property associated with arterial walls, it is interesting to recall the pre-Lister vitalistic view that in some way the arteries kept the blood fluid. Certainly, platelets adhere easily to any particle or surface, with the unique exception of the vascular endothelium. Generation of PGX by vessel walls could be the biochemical mechanism underlying their ability to resist platelet adhesion. Indeed, platelets attempting to stick to vessels may be releasing PGG_2 or PGH_2 which is then used by the vessel to generate PGX which prevents the platelets from sticking. Plaque formation on the arterial wall could hinder access of platelet endoperoxides to the PGX-generating system (or of any continuously formed PGX to the platelets) thereby enabling platelet deposition to occur.

PGX may also have other properties. It is well known that platelets repair lesions in the vascular endothelium[20]. An infusion of a small number of platelets is sufficient to restore vascular integrity in the damaged vessels of thrombocytopenic animals[21,22]. Studies of ultrastructure have revealed that platelets can be assimilated into the vascular endothelium or even incorporated into endothelial cells[23,24]. Biochemical cooperation between platelets and vascular endothelium in the generation of PGX could well contribute to the repair of vascular endothelium.

The fact that PGX is locally generated in the arterial wall and can relax arterial smooth muscle also suggests that it plays a part in the local control of vascular tone. A deficiency of PGX could, therefore, underly some forms of hypertension. It will be interesting to find out whether other tissues can also generate PGX and what other biological properties are associated with this class of compound.

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letters to nature

A secular relativistic change in the period of a binary pulsar

THE discovery of the first binary radio pulsar PSR1913+16 by Hulse and Taylor[1] stimulated an interest in using relativistic effects to calculate more details of this system.[2-4] It was shown, for example, that observations of the apsidal motion could allow the masses of the pulsar and its companion to be calculated. There are many mechanisms which could cause a secular change in the observed period of a binary pulsar (for example, mass exchange magnetic friction, electromagnetic radiation). Here we would like to discuss another possibility related to a change of orbital parameters of a pulsar in a binary system and we also estimate the effect of the emission of gravitational radiation on the period of the binary.

Let the intrinsic period of a pulsar be P_0, its orbital velocity v and the mass of the central star M_0; then the observed period P, neglecting the relative motion between the centre of mass of the binary system and the observer, is given by

$$P=\frac{P_0}{(1-(v/c)^2)^{1/2}(1-(2GM_0/c^2a))^{1/2}} \quad (1)$$

where a is the semi-major axis of the relative orbit, G the gravitational constant and c the velocity of light. For simplicity we will assume that the pulsar moves in a circular orbit. Using Kepler's law to calculate the orbital velocity of the pulsar and keeping only first-order relative corrections, we get

$$P=P_0\left(1+\frac{GM_0}{c^2a}\frac{3M_0+2M_p}{2(M_0+M_p)}\right) \quad (2)$$

where M_p denotes the mass of the pulsar.

Let T be the period of the binary system, then

$$\langle P\rangle=\frac{1}{T}\int_0^T P\mathrm{d}t$$

is the mean period of the pulsar over the period of the binary system. Assuming that there is no mass exchange and M_0 and M_p are constant for the relative time variation of the period we obtain

$$\frac{\dot{P}}{\langle P\rangle}=-\frac{3M_0+2M_p}{2(M_0+M_p)}\frac{GM_0}{c^2a}\frac{\dot{a}}{a}$$

From Kepler's law it follows that when the masses of the components are constant, the relative time variation of the semimajor axis is related to the time variation of the period of the binary system by

$$\frac{\dot{a}}{a}=\frac{2}{3}\frac{\dot{T}}{T} \quad (4)$$

so finally we have the relationship

$$\frac{\dot{P}}{\langle P\rangle}=-\frac{3M_0+2M_p}{3(M_0+M_p)}\frac{GM_0}{c^2a}\frac{\dot{T}}{T} \quad (5)$$

Recently, Taylor *et al.*[5] obtained more accurate values of P and T for the pulsar PSR1913+16, giving $\dot{P}/\langle P\rangle=1.49\times10^{-16}$ and $\dot{T}/T=7.1\times10^{-15}$. Substituting probable values of masses $M_0=1.41M_\odot$ and $M_p=1.41M_\odot$ and taking $a=9.89\times10^{10}$ cm, for a ratio between $\dot{P}/P$ and $\dot{T}/T$ we obtain 1.75×10^{-6}. It therefore follows that for the pulsar PSR1913+16 the change in the period is caused by some mechanisms other than the change in orbital parameters.

Using equation (5), in principle, one should be able to detect the change in the period of a binary pulsar caused by the emission of gravitational waves. Landau and Lifshitz[6] calculated the change in semimajor axis of a binary system caused by the emission of gravitational radiation and obtained

$$\frac{\dot{a}}{a}=-\frac{64G^3M_0M_p(M_0+M_p)}{5a^4c^5}$$

substituting this in (3) we have

$$\frac{\dot{P}}{\langle P\rangle}=\frac{32G^4M_0^2M_p(3M_0+2M_p)}{5c^7a^5}=\frac{2}{5}\left(\frac{R_g}{a}\right)^2\frac{r_g}{a}\left(3\frac{R_g}{a}+2\frac{r_g}{a}\right)\frac{c}{a} \quad (7)$$

where R_g is the gravitational radius of the central star and r_g is that of the pulsar. For PSR1913+16 we get $\dot{P}/\langle P\rangle=1.9\times10^{-22}\,\mathrm{s}^{-1}$, a value which is unobservably small. In a favourable situation, however, with more massive stars forming a compact binary system, the change in the period of the pulsar should be observable. Let us take, for example, a binary system of two stars, one of which could be a black hole, of masses $M_0=15M_\odot$ and $M_p=2M_\odot$ moving in a circular orbit with $a=2\times10^{10}$ cm, then $\dot{P}/\langle P\rangle=6.25\times10^{-16}\,\mathrm{s}^{-1}$ which should be observable with present-day technology.

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Improved upper limits of gravitational deflection of polarised radiation

RECENTLY Harwit *et al.*[1] established upper limits for any polarisation dependence in the bending of radio waves by the Sun's gravitational field. Any differential angular splitting between orthogonal polarisations was shown by VLBI techniques, and from the constancy of the Stokes parameters in the carrier wave from a spacecraft, to be $<10^{-3}$ and 10^{-6}, respectively, of the total bending. Since the latter technique is not sensitive to a splitting in the tangential direction, whereas interferometry is, development of both methods is important. These experiments form a unique test of the equivalence principle[1].

We report here further interferometer measurements. Fomalont and Sramek[2] have measured the total bending of microwave radiation by the Sun's gravitational field. In this paper we search for any polarisation splitting based on a separate analysis of Fomalont and Sramek's data taken with the four-element interferometer of the National Radio Astronomy Observatory (NRAO). For our analysis fringe phases in orthogonal circular polarisations were kindly provided by these investigators. They observed three small diameter radio sources, 0116+08, 0119+11, and 0111+02, at 2,695 and 8,085 MHz with the 35-km intermediate baseline interferometer, as the Sun passed close to their line of sight.

Although the baseline is nearly two orders of magnitude smaller than the baseline of our previous VLBI measurements, the present experiment has produced improved upper limits to the differential deflection. There are two main factors responsible for this improvement. First, the increased phase stability of the NRAO interferometer, together with the higher correlated flux densities at this angular resolution allow a significant increase in signal-to-noise ratio. Second, we were able to observe the sources at smaller solar elongations: 0116+08 was observed to within $<1°$ of the Sun, 0119+11 to within nearly 3°, and 0111+02 to within $<7°$.

The method of analysis was essentially the same as that employed in the VLBI experiment. A fringe phase difference, $\Delta\varphi$, between the orthogonal circular polarisations, of the form

$$\Delta\varphi=G(\rho)[(B_x\sin A-B_y\sin\delta\cos A)\sin H-(B_x\sin\delta\cos A+B_y\sin A)\cos H+B_z\cos\delta\cos A]+\text{constant}$$

was sought, where B_x, B_y, and B_z are the baseline components in wavelengths, A is the position angle of the splitting, H is the

Table 1 Upper limits to the polarisation splitting

		Upper limits on K ($\rho = 1°$) (95% confidence)			
		Tangential splitting		Radial splitting	
n	Frequency (MHz)	$K_{anticlockwise}$ (10^{-3} arc s)	$K_{clockwise}$ (10^{-3} arc s)	K_{out} (10^{-3} arc s)	K_{in} (10^{-3} arc s)
1	2,695	70	95	70	60
	8,085	35	65	30	50
2	2,695	110	130	95	75
	8,085	90	70	60	60
3	2,695	165	195	90	50
	8,085	110	60	45	35

Upper limits to the polarisation splitting are given at a solar elongation of 1°. Clockwise tangential splitting is defined as that seen by an observer in which right circular polarisation appears rotated clockwise about the Sun away from left circular polarisation. In outward radial splitting right circular polarisation appears at greater solar elongation than left circular polarisation.

hour angle, and δ is the declination. Any angular splitting, $G(\rho)$, would be expected to depend on the solar elongation, ρ. Simple power law radial dependence of the form $G(\rho) = K\rho^{-n}$ ($n = 1, 2, 3$) was assumed.

No polarisation splitting was observed. Ninety-five per cent confidence upper limits were obtained by calculating χ^2 for various assumed values of the splitting. These upper limits are given in Table 1 for both frequencies, for both tangential and radial splitting, and the different values of n. Three intermediate length baselines were obtained by correlating signals from the 45-foot antenna with those from the three 85-foot antennae. These results were not statistically independent and the baseline yielding the smallest upper limits was chosen. The upper limits in Table 1 are ~50–100 marc s at a solar elongation of 1°.

With observations at low solar elongations, the present observations yield improved upper limits for any radial dependence as strong as ρ^{-2} or stronger. (The previous upper limits of Harwit *et al.*[1] were established for solar elongations of 5° and 7.8°, not 5 R and 7.8 R, as given in Table 1 of ref. 1.)

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Origin of Olympus Mons Escarpment by erosion of pre-volcano substrate

OF all the large shield volcanoes of Mars, Olympus Mons is unique in being fringed by a nearly continuous scarp rising 1–4 km above the surrounding terrain[1,2] (Fig. 1). We present evidence that the scarp was caused by preferential erosion of fragmental cratered terrain material previously adjacent to, and at present underlying the shield volcano. Removal of this material caused undercutting and collapse of the shield flanks, producing the observed terraces and scarps.

A previous interpretation for the scarp was prompted by the recognition that basaltic lava flows which probably form the flanks of Olympus Mons are very resistant to any aeolian erosion[3]. King and Riehle[4] proposed that the volcano has a layered structure with basaltic lava flows capping a base of ash flow tuff deposits which would be readily erodable by aeolian processes. Such an origin for the scarp is unlikely, since volcanoes with basaltic tops and acidic bottoms are not produced typically by terrestrial igneous processes[5]. Rather, it is common for differentiation within magma chambers of terrestrial basaltic volcanoes to result in late stage eruptions of small volumes of more silicic lavas, as at Mauna Kea, Hawaii[6].

Undercutting such as apparently initiated the formation of the Olympus Mons scarp commonly occurs when an upper resistant layer is left unsupported through the removal of a less competent substrate. It is here proposed that the Olympus Mons substrate is a layer of fragmental rubble (megaregolith) resulting from repeated fracturing and brecciation of the surface by intense meteoritic bombardment. On the Moon, the cratered terrain megaregolith is 2–4 km in thickness[7], and a similar thickness may exist on Mars.

Much of the northern hemisphere of Mars is a smooth lava plain with a lower concentration of craters than the rest of the planet. We propose that this is not caused by any fundamental difference in crustal formation, but rather that the original cratered terrain has been stripped off in some erosional event, and the exposed surface veneered with lavas. It is not understood how this happened, nor why the destruction was limited to the northern hemisphere of Mars. Nonetheless, cratered terrain was removed leaving scattered remnants, and the edge of the cratered terrain is presently undergoing erosion[8]. The northern lowlands of Mars are 2–3 km lower than the southern cratered terrain. This difference in elevation is nearly the same as the height of the Olympus Mons scarp, and about the same as the lunar/martian megaregolith thickness. This implies that the erosional event removed the megaregolith only and did not excavate and remove large quantities of well-consolidated basement rock.

In this model the initial Olympus Mons lavas erupted upon cratered terrain, armouring it against later erosion. Removal of the cratered terrain megaregolith led to the undercutting of Olympus Mons lavas and to the formation of the scarp. Thus, the scarp itself is a relic of earlier erosion and not caused by the present erosional regime which only modifies the original structure. This would account for the previously puzzling contrast between the severe erosion of the scarp and the minor erosion higher up on the flanks of Olympus Mons. In some places (for example, the SW flank) later lavas from Olympus Mons flowed over the scarp, re-establishing the original slope. On

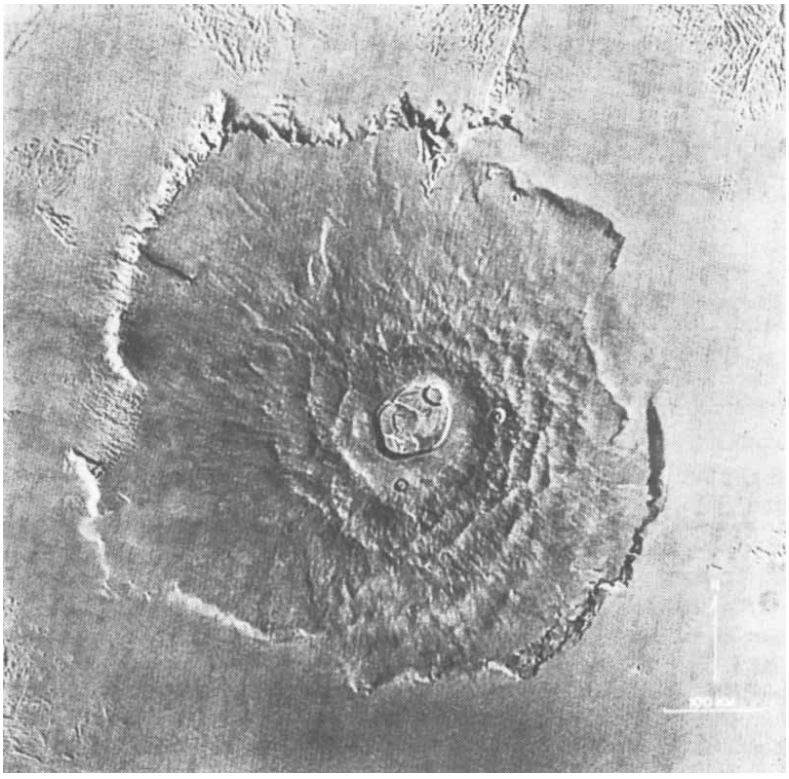

Fig. 1 Complex relationships between Olympus Mons shield volcano and the surrounding lava plain are indicated by the embayment of the eastern flank of the shield and by mantling of the south-west escarpment and plains by shield lavas. The photograph has been computer rectified, enhanced and mosaicked at the Image Processing Laboratory of the Jet Propulsion Laboratory by Karl Blasius and Jim Soha.

the other hand, it seems that lavas from the surrounding plain have lapped on to the eastern flank of the volcano. Thus, Olympus Mons spans the time interval from before the onset of the erosional event to a period more recent than the emplacement of the youngest lavas of the surrounding plain. From crater counts[9,10] this interval is estimated to have been 2×10^8–2×10^9 yr, corresponding to a minimum average eruption rate of $\sim10^{-3}$–10^{-2} km^3 yr^{-1}. For comparison, the eruption rate averaged over the past 70×10^6 yr of the Hawaii–Emperor chain is 1.5×10^{-2} km^3 yr^{-1} (ref. 11).

Neither the volcanoes of the Tharsis plateau nor those of Elysium have scarps such as bound Olympus Mons. According to the hypothesis developed here scarps would not form unless the ancient cratered terrain substrate was eroded away after the initial armouring eruptions. The geological map of the Tharsis region[12] shows 'hilly material', interpreted as remnants of cratered terrain, extending out from under the Tharsis volcanoes. This demonstrates that the cratered terrain was not completely destroyed during the erosion episode and thus, the Tharsis volcanoes were not undercut and did not develop scarps. Similar circumstances occur in the Elysium region. The Elysium dome is fringed by irregular rounded hills[12] which seem to be the erosional remnants of cratered terrain. Apparently, the ancient cratered terrain was not completely removed and thus scarps were not formed in this region.

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Correlation of Martian surface heights with latitude of polar hood boundaries

A COMPARISON of Earth-based photography with topographic data from Mariner 9 reveals that the winter polar hoods on Mars tend to extend further towards the equator at lower topographic heights than at higher topographic heights. The correlation between latitude of the hood boundary and topographic heights is shown in Figs 1 and 2. In Fig. 1, contour lines derived from a map by Christensen[1] have been superimposed on the Lowell Observatory map of Martian Albedo Features and Topography[2]. To the author's knowledge, the Christensen map is the only published map that includes contour lines up to 65°N. The dashed lines represent the mean boundary of the polar hoods during twelve apparitions between 1905 and 1958, as determined from a study of Lowell Observatory's historical photographic collection. The southern hood boundary is in close agreement with a map by Capen[3] that was made from measurements of more recent apparitions (1962–68), while the northern boundary agrees well with Mariner imaging[4]. In Fig. 2, the latitude of the hood boundaries (at 10° intervals in longitude) is plotted as a function of topographic height. The dashed line was fitted to the southern latitude points by least squares. The north hood points were then shifted along the ordinate until their average value fell on the dashed line. An equally good, if not better, correlation is found if one plots the latitude of the boundary of the southern hood against atmospheric pressure from the work of Conrath *et al.*[5]. The hood is found to extend farther north over areas of higher pressure than over areas of lower pressure. Comparable atmospheric pressure data are not available for the Northern Hemisphere.

The explanation of the phenomenon reported here is not

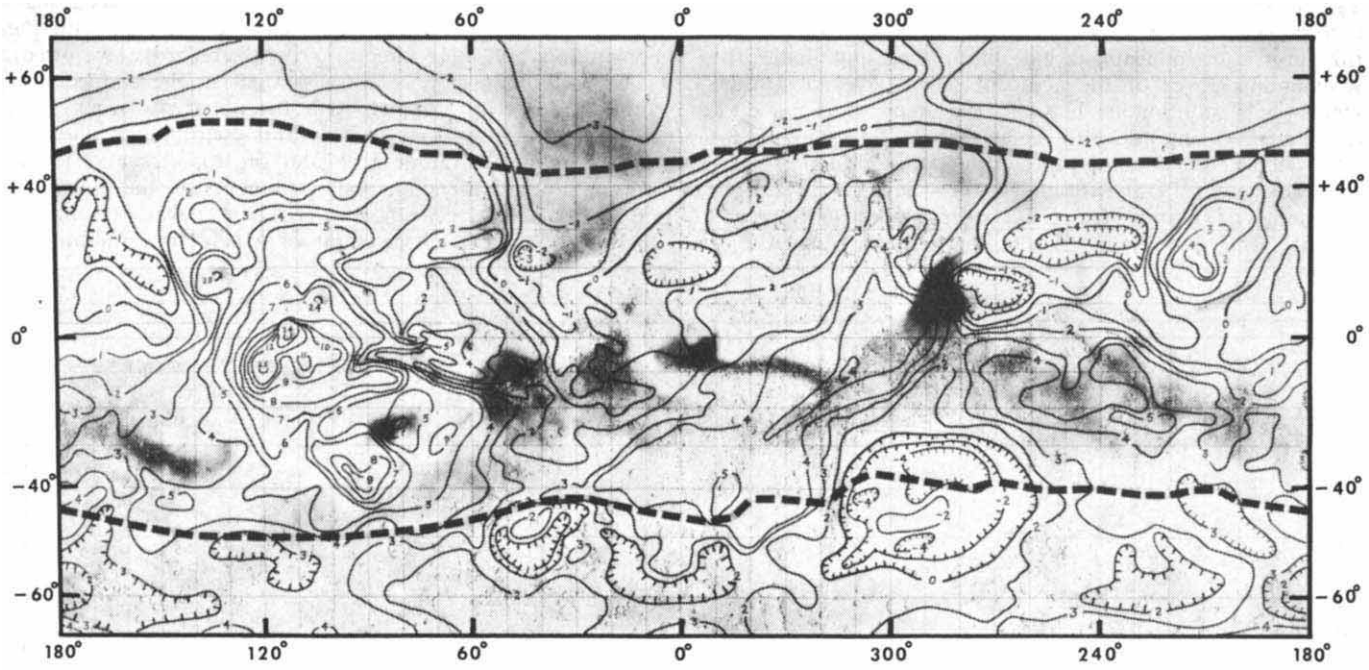

Fig. 1 Averaged boundaries for Martian polar hood clouds during northern and southern winters. The seasonal positions of Mars in its orbit that were used are L_s 315°–344° for the north and L_s 135°–164° for the south. The contour lines give heights in kilometres and have been redrawn by C. F. Capen from Christensen[1] on to the Lowell Observatory map of Martian Albedo Features and Topography[2].

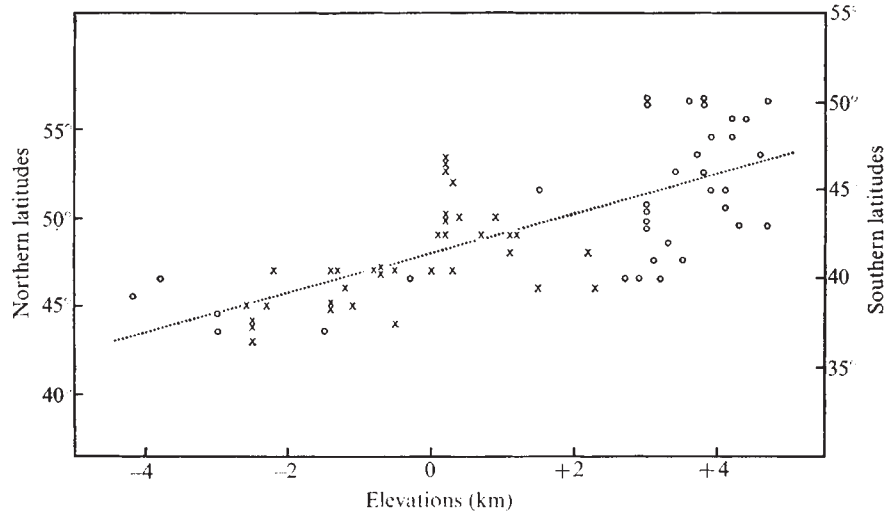

Fig. 2 Correlation of surface heights with areographic latitude of the averaged boundaries of Martian polar hood clouds, measured on red- and green-filter photographs from the Lowell collection, taken from 1905 to 1958. The latitudinal scales have been offset to compensate for systematic differences between northern and southern winter hoods. The dashed line was fitted by least squares to the southern hood data. ×, North hood (L_s = 315°–344°); ○, South hood (L_s = 135°–164°).

yet completely clear. Briggs and Leovy[4] have, however, argued that the hood clouds in the zone from 40 to 50°N must be water ice, while Farmer[6] has suggested that the bulk of the water vapour will be found in the lowest atmospheric layers.

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A granite cliff deep in the North Atlantic

WE report here preliminary results from the detailed sampling of granitic basement at a depth of 4,000 m at the lower edge of the Armorican continental margin near 48°N and 12°W. We consider that we have sampled here the extreme lateral limit of the continental crust where local exposures emerge from under a cover of Cainozoic, Mesozoic and probable Palaeozoic sediments.

The Goban Spur[1], part of the deep continental margin off France and the UK, lies between the Celtic shelf and the Porcupine abyssal plain ~ 550-km West of Brest and Land's End (Fig. 1). A seismic profile of the continental slope in this area shows a succession of three main 'gradins' (steps) with intermediate sedimentary basins (Fig. 2). The westernmost and deepest of the steps has a steep south-western slope Granite Cliff 4,000 near the 4,000-m isobath.

Twenty km further to the south-west is the marginal hill or high named by us Menez Bihan. At each of the three steps the substratum appears as an outcrop, passing under the sedimentary cover of the adjacent basin. The substratum seems to be continuous between the steps; it is 3-s d.t.t. (double travel time) deep to the east of the first step and 8-s d.t.t. deep at the foot of the continental slope (Fig. 2).

In December 1975, with the RV Le Suroît, we dredged fragments of granite from the steep slope we now call Granite Cliff 4,000 (Fig. 2). On the side of King Arthur Canyon, at a depth of 3,000 m, we dredged a pebble containing fossil material identified as Devonian. To investigate further these findings, and to make sure that the granite dredged was not erratic, we went back to the area in February 1976 with the RV Jean Charcot.

We made a bathymetric survey to clarify the relationship between Granite Cliff 4,000 and Menez Bihan and then moored three acoustic beacons on the bottom: two at the foot of the cliff and one near the top (all within the circle in Fig. 1). The distance between beacons was ~ 6,000 m. On board, on a plotting table, the track of the ship and the track of the dredge (or camera) on the bottom were then followed in real time with a precision of ~ 30 m. In this manner it is possible to visualise the work of the dredge on the bottom and, in combination with the tensiometer, to determine with precision the position of breaking-off of the rocks. For the seafloor photography, we used both a troika (a toboggan that slides on the bottom) and a camera frame attached to the cable just ahead of the dredge.

The dip of the slope of Granite Cliff 4,000 reaches at least 30° (the maximum slope which can be detected with the conventional 12-kHz precision depth recorder we used). The base of the slope is very straight in the studied area and this linearity, coupled with the apparent steepness of the slope, suggests that it is a fault escarpment. The foot of the slope is 4,150-m deep and the top lies at 3,150 m. Photographs of the slope show sediments on the lower and upper parts. In between, the sediment cover is thin and there are round outcrops, some of which show fractures.

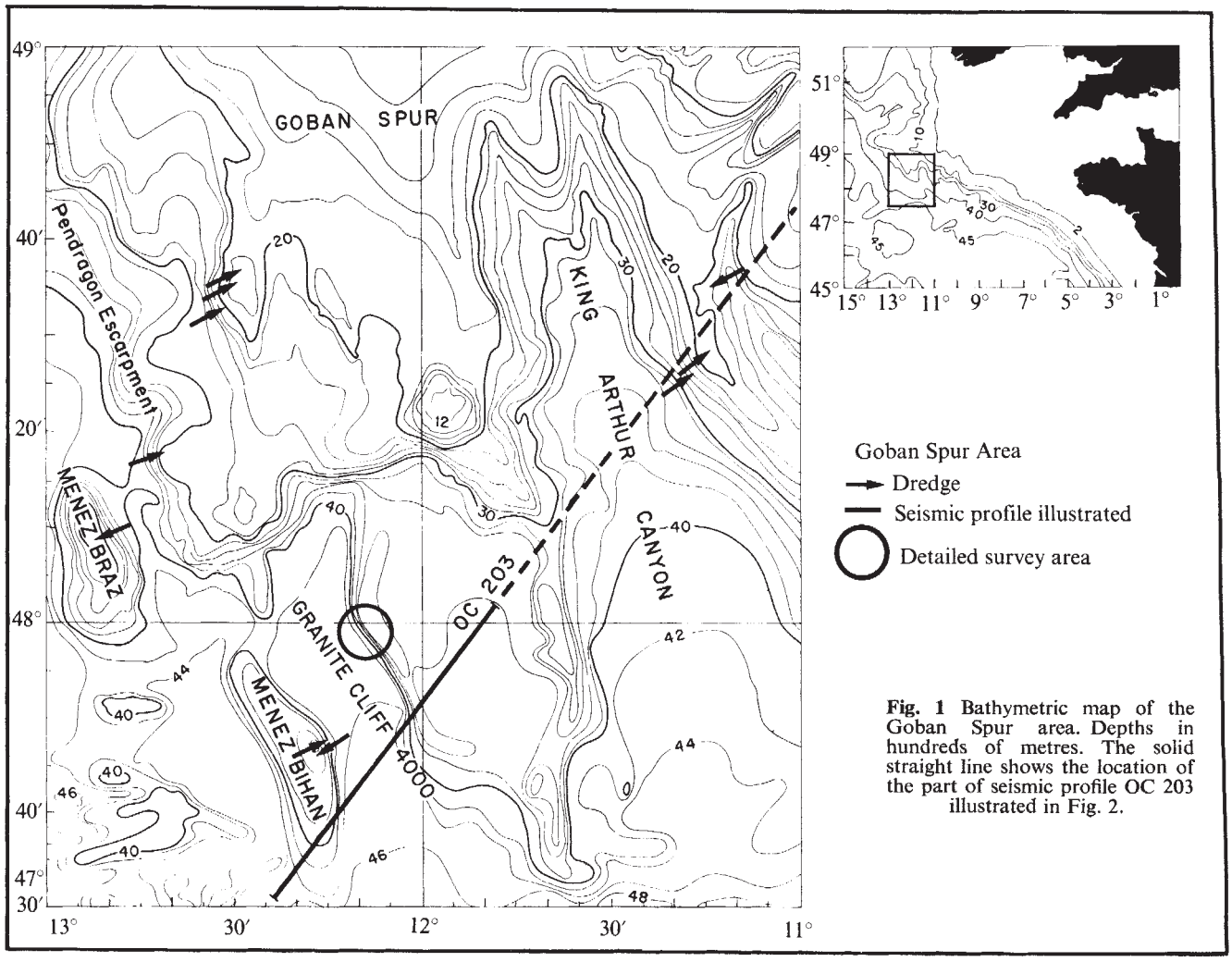

Fig. 1 Bathymetric map of the Goban Spur area. Depths in hundreds of metres. The solid straight line shows the location of the part of seismic profile OC 203 illustrated in Fig. 2.

Four dredgings with the acoustic navigational control were made and in each dredge we found blocks of granitic rock. Their appearance and the dredge-wire tensiometer records indicate that some of the blocks were torn off outcrops, and support the evidence from the seafloor photographs that we are not dealing with ice-rafted erratics, except perhaps for a few associated pieces of dark or light granulite, the autochonous origin of which is still in doubt. In addition, the presence of all the rocks found can be reasonably associated with the setting in which they occur.

The granitic blocks are more or less weathered and are partially covered with ferromanganese crusts up to 12-cm thick. They are granodiorites, tending to quartz diorites. The minerals present are quartz with undulating extinction, biotite (abundant), andesine (abundant, automorphic and zoned), potassic feldspar (rather scarce), apatite, zircon and opaque minerals (J. Didier, personal communication). The fabric is clearly directional, and the biotites are slightly curved.

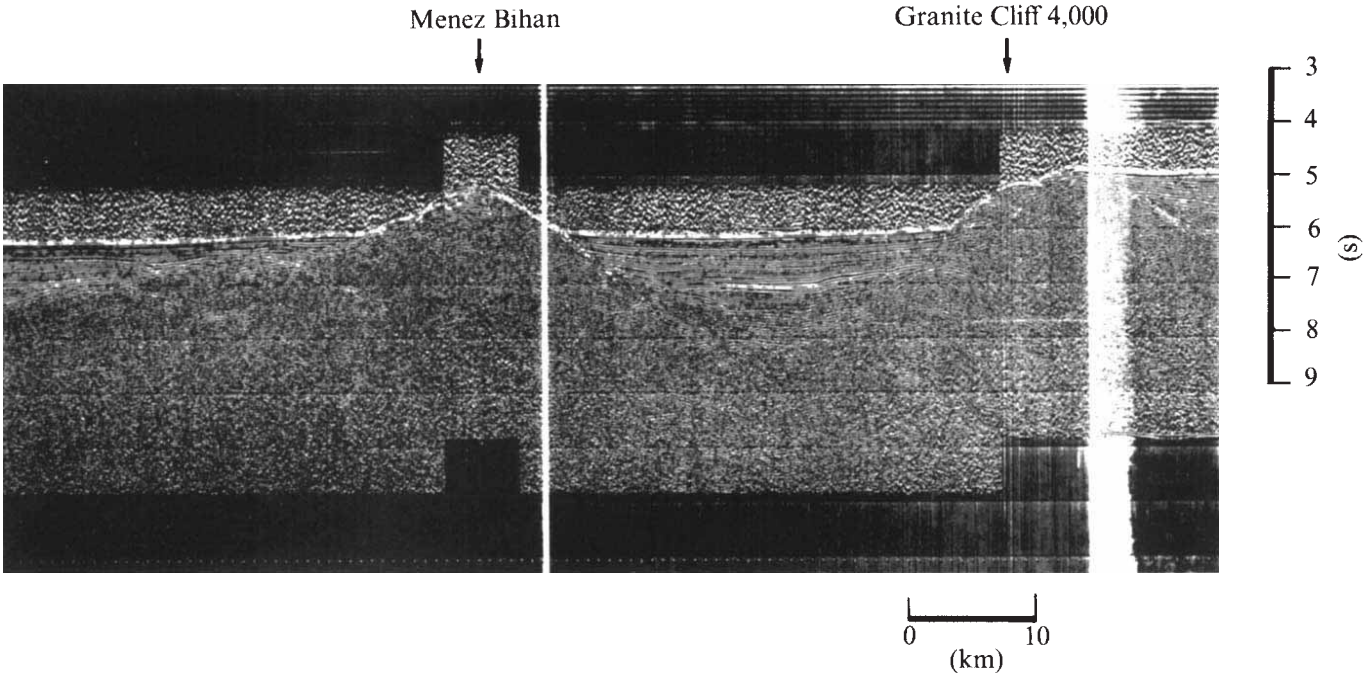

Fig. 2 Seismic profile OC 203 made by the RV Florence. Location of the profile is shown on Fig. 1. Vertical exaggeration is ×5.

Similar rocks possibly a little more altered, were dredged on the flanks of Menez Bihan (Fig. 2). The apparent continuity between Menez Bihan and Granite Cliff 4,000 (Fig. 2) is thus demonstrated. On geomorphic grounds (Fig. 1) it seems probable to us that Menez Braz to the north-west is also granitic, but we obtained no samples because of the considerable thickness of sediment there.

In three areas we dredged fragments of old sedimentary cover lying above the granitic substratum. From the flanks of Menez Bihan we collected a fragment of quartzitic sandstone. The remainder of the dredge-haul was entirely granitic Granite Cliff 4,000 yielded numerous samples of limestones some of them of possible Mesozoic age. On the Pendragon escarpment (Fig. 2), at a depth of between 3,000 and 2,000 m, the dredge recovered no granite, but a few metamorphic rocks and a rich sample of old sedimentary rocks: fine green sandstones, marly chalk with veins of calcite, grey claystone, sedimentary breccias and quartzite. No fauna has been found on preliminary examination, but we speculate that the rocks represent Palaeozoic or even older sediments.

The dredging of 'in place' granitic rocks at 4,000 m is exceptional and has important implications. In geometric reconstructions of the early opening of the North Atlantic, for example, many authors[2] have used a shallower depth (1,000 to 2,000 m) and hence a more easterly limit, for the edge of the continental crust. The maximum depth of the granite is evidently even greater than 4,000 m. Judging from the seismic profile (Fig. 2), the probable continuity of the granitic substratum between Granite Cliff 4,000 and Menez Bihan lies at a depth of 8 s d.t.t. (between 6,500 and 7,500 m).

To the south-west of Menez Bihan lies a low hill above the 4,000-m water depth (Fig. 1). Seismic profiles show that the basement of this hill is rough: quite different in character from that of Menez Bihan. Magnetic records show that the east flank of the low hill marks the beginning of a sharp anomaly (500×10^{-9} T) whereas the magnetic profile on the continental side of the hill is relatively flat. According to C. Williams (personal communication), anomaly 34 (~ 85 Myr) passes close by. The hill does not seem to have the acoustic character of an intrusion, and may indeed mark the contact between oceanic crust and continental crust[3].

The granodioritic character of the granitic massifs we sampled suggests that they are orogenic granites, presumably of the intrusive type and of deep origin. It is difficult to envisage their emplacement during early rifting (90–100 Myr). None of the granites is alkaline and they are neither porphyritic nor strongly deformed. On the other hand, they may well be late generation (post-phase 2) granodiorites of Hercynian age (J. Didier, personal communication). They may represent intrusions in a granulitic basement which would explain the rocks belonging to the granulitic facies that we dredged from Granite Cliff 4,000 and from Menez Bihan. The basement underlying the continental slope itself is probably not made up of granodiorites. The latter usually appear as intrusions in the Hercynian chains (for example, the Armorican massif).

The oldest sedimentary rocks are epimetamorphic with at least two phases of deformation (folding). They resemble the Palaeozoic and Upper Precambrian (Brioverian) rocks of the Armorican massif (M. Gravelle, personal communication). We interpret this evidence as further support for arguing that the basement underlying the continental margin east of Menez Bihan is probably the older Hercyninan basement (granulite with granodiorite intrusions).

The direction of Granite Cliff 4,000 is similar to the Pendragon Escarpment (N 30° W) and may be related to Hercynian faults[4]. During the rifting phase of the North Atlantic, normal faulting associated with the subsidence of the continental blocks probably reactivated these old features. During the initial uplift phase, Menez Bihan would—in most models—have been on top of the uplifted crust and would consequently have been deeply eroded. Thus it seems reasonable that we dredged granite and Mesozoic limestone on Granite Cliff 4,000 and no Palaeozoic sediments. The upper part of Pendragon Escarpment, further away, was not, however, eroded to such an extent. Subsequently, thermal processes led to inversion of relief, with normal faulting along the Hercynian zone of weakness and subsidence of the continental margin.

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Infrared polarisability of hexagonal ice

THE limiting high frequency polarisability, α, of a solid is the algebraic sum of the optical, or electronic, polarisability, α_e, of the ions or molecules, and the infrared polarisability, α_{IR}, caused by the displacement, or vibration, of ions or molecules within the lattice. For most solids both α_e and α_{IR} are independent of volume and temperature, but for a number of ionic solids and for polymorphs of ice, α_{IR} is not so. We describe the temperature dependence of α_{IR} of hexagonal ice, and discuss it in terms of contributions from anharmonic effects.

For an isotropically polarisable material, α is given by the Clausius–Mossotti relationship

$$\frac{4}{3}\pi N\alpha = V\left(\frac{\varepsilon_\infty - 1}{\varepsilon_\infty + 2}\right) \quad (1)$$

where N is Avogadro's number, V the molar volume and ε_∞ the limiting high frequency relative permittivity of the orientation polarisation. V for H_2O ice was calculated for a density of 0.9169 Mg m^{-3} at 270 K, and the average expansivity[1–5] is given in Fig. 1. V for D_2O ice was calculated for a density of 1.0175 Mg m^{-3} at 270 K and Dantl's[3] expansivity data. ε_∞ of H_2O ice for 2–77 K is taken from Gough's data[6] and for 77–273 K from our own[7]. ε_∞ of D_2O ice is known only above 77 K (ref. 8).

α_e for an H_2O molecule in ice is 1.5 Å^3 (ref. 9), and α_e for a D_2O molecule in the liquid phase, calculated from the Lorentz–Lorenz equation, is 1.46 Å^3 (ref. 8), which value is likely to be the same in D_2O ice. α_{IR} ($= \alpha - \alpha_e$) calculated from these values of α and α_e for both the ices is plotted against temperature in Fig. 2, which shows that the infrared polarisability of ice increases with temperature.

$$\alpha_{IR} = \alpha_{0,IR} + AT^2 \quad (2)$$

where $\alpha_{0,IR}$ is the infrared polarisability at O K and A is an empirical constant. The values of $\alpha_{0,IR}$ and A are 1.64 Å^3 and 2.03×10^{-6} Å^3 K^{-2} for H_2O and 1.49 Å^3 and 2.95×10^{-6} Å^3 K^{-2} for D_2O ice. The T^2 increase in α_{IR}, which seems unique to hexagonal ice, is attributable to the anharmonicity of mechanical and electrical vibrations of H_2O molecules, as a consequence of which, the force constant of molecular vibrations becomes amplitude and temperature dependent. $\alpha_{0,IR}$ can then be interpreted as the infrared polarisability if the lattice vibrations were entirely harmonic, as is anticipated at 0 K.

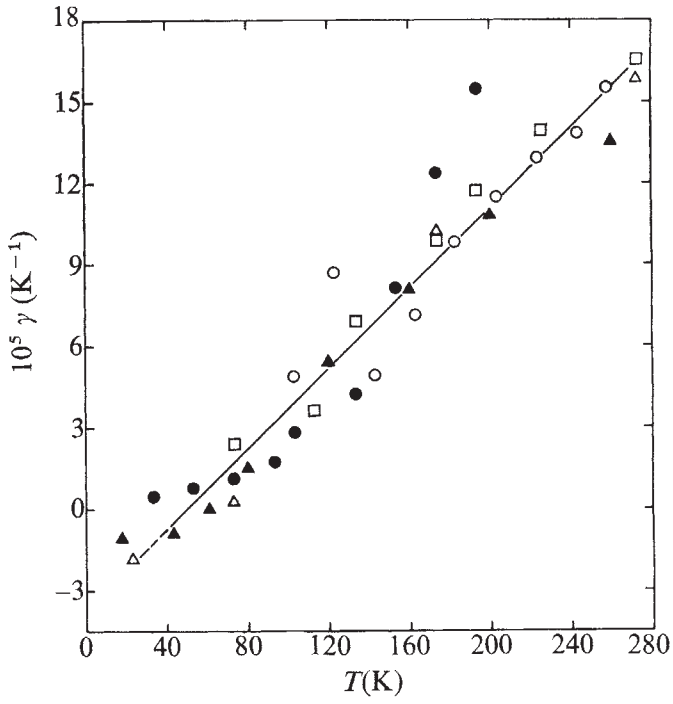

Fig. 1 The isobaric expansivity, γ, of H_2O ice plotted against temperature. The values were interplotted from, Δ, Jacob and Erk[1]; ■, Powell[2], ▲, Dantl[3] for bulk expansion; and are as given by ○, LaPlaca and Post[4]; and ●, Brill and Tippe[5] for lattice constant expansion. The straight line between 30 and 273 K is the 'best' curve, in lieu of a least square fit which seems inappropriate because numerical data from various authors are lacking. Results from refs 2, 4 and 5 have been converted to values for polycrystalline ice.

The anharmonicity of lattice vibrations is also reflected in the temperature dependence of heat capacity and expansivity, and a consideration of the latter is useful in an analysis of the T^2 dependence of α_{IR}. The temperature coefficient of α_{IR} at a constant pressure may be divided into two

$$\left[\frac{\partial\alpha_{IR}}{\partial T}\right]_P = \left[\frac{\partial\alpha_{IR}}{\partial T}\right]_V + \gamma\left[\frac{\partial\alpha_{IR}}{\partial \ln V}\right]_T \quad (3)$$

where γ is the volume expansivity. $(\partial\alpha_{IR}/\partial T)_V$ constitutes the volume-independent temperature effect and $(\partial\alpha_{IR}/\partial \ln V)_T$ the temperature-independent volume effect.

Because of the lack of an adequate theory of anharmonic effects, the exact relation between γ and T is not known. For ice, such a relation is further uncertain because of the great variation between the reported values of its γ, as evident in Fig. 1. In the range, 30–273 K, γ can still be adequately described by the equation, $\gamma = \gamma_0 + mT$, as shown in Fig. 1, although the linear relationship is not anticipated to hold near 0 K because theory requires that γ should be zero at 0 K. For H_2O ice, $\gamma_0 = -3.6\times10^{-5}$ K^{-1} and $m = 7.39\times10^{-7}$ K^{-2}. Substituting for γ in equation (3), one obtains

$$\left[\frac{\partial\alpha_{IR}}{\partial T}\right]_P = \left[\frac{\partial\alpha_{IR}}{\partial T}\right]_V + \gamma_0\left[\frac{\partial\alpha_{IR}}{\partial \ln V}\right]_T + m\left[\frac{\partial\alpha_{IR}}{\partial \ln V}\right]_T T \quad (4)$$

By differentiating equation (2)

$$\left(\frac{\partial\alpha_{IR}}{\partial T}\right)_P = 2AT \quad (5)$$

A comparison of equations (4) and (5) suggests that, unless $(\partial\alpha_{IR}/\partial T)_V$ is a linear function of temperature

$$\left(\frac{\partial \alpha_{IR}}{\partial T}\right)_V = -\gamma_0\left(\frac{\partial \alpha_{IR}}{\partial \ln V}\right)_T$$

and

$$\left(\frac{\partial \alpha_{IR}}{\partial \ln V}\right)_T = \frac{2A}{m}$$

For H_2O ice, using values of A and m given above, $(\partial\alpha_{IR}/\partial \ln V)_T = 5.49$ Å^3 and $(\partial\alpha_{IR}/\partial T)_V = 1.98\times10^{-4}$ Å^3 K^{-1}.

The contribution to $(\partial\alpha_{IR}/\partial T)_P$ in equation (3) from a temperature change alone, $(\partial\alpha_{IR}/\partial T)_V$, is positive for ice, as it is for ionic crystals of NaCl structure[10], but the contribution from a volume change, $\gamma(\partial\alpha_{IR}/\partial \ln V)_T$, is negative at $T < 50$ K and positive at $T > 50$ K, because γ changes sign near 50 K (Fig. 1). Near 273 K, $\gamma(\partial\alpha_{IR}/\partial \ln V)_T$ increases to nearly 4 $(\partial\alpha_{IR}/\partial T)_V$. Thus the difference between the respective temperature variation of α_{IR} at constant volume and constant pressure is substantial near 273 K and all of the increase in α_{IR} (and ε_∞) with temperature results from the associated volume change rather than to any explicit dependence on temperature.

As the nonlinear effects of anharmonic terms in the intermolecular potential, responsible for expansivity, are anticipated to disappear near 0 K, $\gamma \to 0$ as $T \to 0$ K. Therefore $(\partial\alpha_{IR}/\partial T)_V \to 0$ at 0 K as does $(\partial\alpha_{IR}/\partial T)_P$. A complete analysis of the interesting features of α_{IR} in the low temperature range would require accurate values of γ, $(\partial\alpha_{IR}/\partial T)_V$ and $(\partial\alpha_{IR}/\partial \ln V)_T$, not available at present. The validity of the relation between α_{IR} and T^2 for ice, however, is not likely to be significantly affected by a possible negative value of γ below 50 K, for the data points below 50 K lie in a relatively small range of T^2, as shown in Fig. 2.

The pressure dependence of α_{IR} and ε_∞ of ice can be predicted from

$$\left[\frac{\partial \alpha_{IR}}{\partial P}\right]_T = -\beta\left(\frac{\partial \alpha_{IR}}{\partial \ln V}\right)_T \qquad (6)$$

where β is the compressibility. For H_2O ice $\beta = 12\pm1$ Mbar^{-1} (ref. 11) at $20 < T < 273$ K, and therefore $(\partial\alpha_{IR}/\partial P)_T = -65.9$ Å^3 Mbar^{-1}.

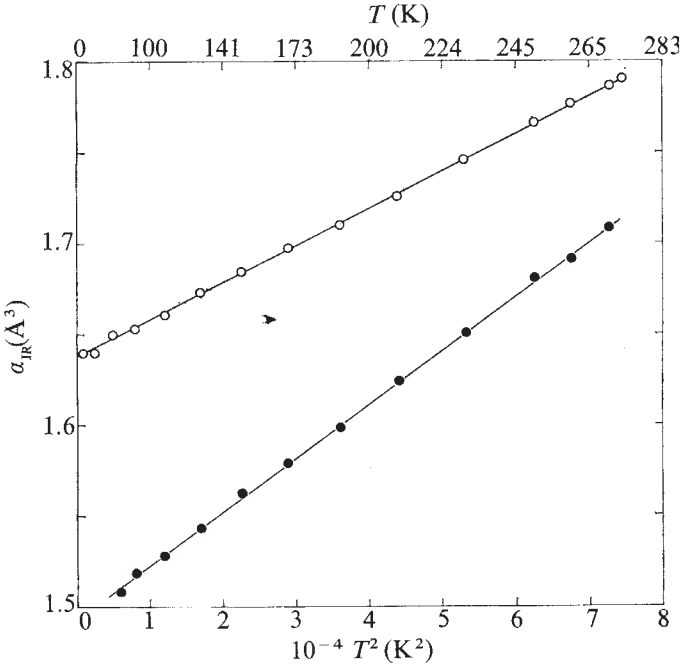

Fig. 2 The infrared polarisability, α_{IR}, of ○, H_2O and ●, D_2O ice plotted against the square of temperature. The relation between α_{IR} and T^2 is little affected by the variation in the reported values of γ seen in Fig. 1.

By differentiating equation (1) with respect to pressure, one obtains

$$\left(\frac{\partial \varepsilon_\infty}{\partial P}\right)_T = \frac{1}{3}(\varepsilon_\infty+2)(\varepsilon_\infty-1)\left[\beta+\frac{1}{\alpha_{IR}}\left(\frac{\partial \alpha_{IR}}{\partial P}\right)_T\right] \qquad (7)$$

At 270 K, $\varepsilon_\infty = 3.19$ (ref. 7), and therefore from equations (6) and (7), $(\partial\varepsilon_\infty/\partial P)_T = -94$ Mbar^{-1}. Thus, both the ε_∞ and α_{IR} of ice are anticipated to decrease by $\sim 3\%$ kbar^{-1} near 270 K.

γ (ref. 3) and β (ref. 11) for H_2O and D_2O ice are the same and at 270 K, $\varepsilon_\infty = 3.09$ (ref. 8) for D_2O ice. The analysis of α_{IR} for D_2O ice thus gives: $(\partial\alpha_{IR}/\partial \ln V)_T = 7.98$ Å^3, $(\partial\alpha_{IR}/\partial T)_V = 2.87\times10^{-4}$ Å^3 K^{-1}; $(\partial\alpha_{IR}/\partial P)_T = -95.8$ Å^3 Mbar^{-1} and $(\partial\varepsilon_\infty/\partial P)_T = -156$ Mbar^{-1}. The decrease in α_{IR} and ε_∞ on application of pressure is, therefore, anticipated to be $\sim 60\%$ more in D_2O than in H_2O ice.

The magnitude of the contribution to ε_∞ from vibrational polarisation is directly related to the negative second moment of the absorptivity, according to the Kramers–Kronig relationship (see ref. 7 for details). It follows that the application of pressure should either increase the frequency of the major absorption bands and/or decrease the absorptivity of the infrared radiation in ice.

The above conclusions are of significance because measurements of both ε_∞ and the infrared spectra of hexagonal ice with pressure are difficult, and because it is widely assumed that the application of pressure should increase ε_∞ of ice.

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The valence of transition metal atoms in metallic alloys

THE 'valence' of transition metal atoms in metallic alloys has long been and still is a source of controversy. On the one hand, there are measurements such as those of Haworth and Hume-Rothery[1] which indicate quite small valences, there are electron concentration rules in numerous Hume-Rothery β and γ phases which indicate 'zero' valences, and there are measurements (for example, Coppens[2]) which even indicate 'negative' valences, whereas on the other hand, melting points, hardness and heats of atomisation indicate much higher valences. In this paper we point out that the valence of a transition metal atom in a metallic alloy cannot usefully be described by a single number. Three parameters are required: the number of outer electrons contributed to partly filled energy bands, the number entering filled bands lying below the Fermi level, and a parameter ($0<\alpha<1$) describing the effectiveness for cohesion of the hybridisation of the filled bands originally derived from d states with those originally derived from s and/or p states.

This is because the valence electrons contributed by the transition metal may become part of two distinct groups which have different properties. The number and properties of electrons in one group—partly filled sheets of Fermi

surface (partly filled energy bands)—can be explicitly measured in suitable cases, whereas the properties of the other group—electrons in filled energy bands lying below the Fermi level—are always seen with those of the other group. Thus the valence of a transition metal atom in a metallic alloy cannot usefully be described by a single number. One parameter is required to specify the number of electrons contributed to partly filled energy bands in the alloy, another to describe those contributed to filled energy bands which have mainly d character. A further parameter, $0<\alpha<1$, is required to describe the effectiveness of the hybridisation of the filled bands originally derived from d states with those originally derived from s (and/or p) states, which ensures that the electrons are cohesive ($\alpha>0$) even though they lie below the Fermi level. Such a description does not seem to be basically different to a valence bond description that considers the formation of hybridised bond orbitals which are derived from d, s and/or p states (see, for example, Pauling[3]; Altman, Coulson and Hume-Rothery[4]), but it does have the advantage when applied to metallic alloys of allowing one to recognise the difference between the numbers of itinerant electrons in partly filled energy bands and those in filled energy bands, which give the differences in physical measurements or estimates of valence referred to above.

Thus it is obvious that in determining the valence of Mn, Fe, Co and Ni from phase boundaries in certain alloys, Haworth and Hume-Rothery[1] were measuring properties controlled by the number of electrons contributed by the transition metals to the partly filled bands. In β and γ brass-type phases where many transition metals are said to be 'zero valent' to satisfy electron concentration rules, the outer electrons of the transition metal atoms are in filled bands below the Fermi level and the valence electrons contributed by the other component are sufficient to provide the required electron concentration in the partly filled bands. This is confirmed by the similarity of the Fermi surface of β-PdIn to those of β-AgZn and β-CuZn, indicating in each case, three electrons in partly filled sheets of Fermi surface in Brillouin zones one and two (Jan, Pearson and Saito[5]). Since indium contributes three valence electrons, the 10 outer electrons of Pd can be regarded as occupying filled bands below the Fermi level, and 'zero valence' is to be interpreted as contributing no net electrons to the conduction bands. Similarly an apparent negative valence of a transition metal is interpreted as retention of some of the valence electrons contributed by the other alloying component(s) in filled bands below the Fermi level, so that the electron concentration in partly filled sheets of Fermi surface (conduction bands) is reduced below the number of electrons contributed by the other alloying component(s).

The melting points of the transition metals and their heats of atomisation generally rise to a maximum in the fifth or sixth Group where the most effective cohesion from the outer electrons, is obtained. Although Fermi surface studies show the noble metals, Cu, Ag and Au, to have only one electron in partly filled bands, the high melting points (1,083 °C for Cu) and the heats of atomisation (81.1 kcalorie per gram atom at 298.15 K for Cu (Brewer[6])) indicate far more cohesion than that obtained from one outer electron (20–30 kcalorie per gram atom in heat of atomisation). This extra cohesion comes from hybridisation of the bands derived from d states with those derived from s and p states so that they contribute to cohesion even though they are full and lie below the Fermi level. The low melting point and the heat of atomisation of Zn (31.2 kcalorie per gram atom at 298.15 K (Brewer[6])), however, correspond to the two outer electrons in partly filled bands and indicate virtually no remaining hybridisation of the d bands to contribute to cohesion (that is $\alpha\sim0$).

In summary, then, if V_i represents the number of outer electrons contributed by the transition metal to partly filled energy bands and V_1 the number contributed to filled energy bands, V_i may, in favourable cases, be determined from measurements of properties sensitive to the electron concentration in partly filled energy bands (Fermi surface, phase boundaries, etc.) and the number of valence electrons contributed by a non-transition element in the alloy. In considering properties such as melting points and cohesive energy, the contributions of both αV_1 and V_i are to be considered. Thus, for example, although electron concentration rules indicate $V_i\sim0$ in β-brasses such as AlCo or AlNi, their high melting points (1,645 and 1,638 °C respectively) indicate a sizeable value of αV_1, which with the three electrons per formula unit contributed by Al, gives the large cohesive energy. The classical chemical valences of the transition metals (2+, 3+, etc.) are normally found in compounds with filled energy bands that are insulators or semiconductors.

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The glass transition temperatures of phosphoric acids

THE vitrification of phosphoric acids is relatively easy, a glass often being formed simply on cooling from the liquid melt. Special procedures, however, are required to crystallise these materials. The glass transition temperature, T_g, is the temperature above which there is relatively rapid molecular motion; below T_g translational motion of molecules is inhibited. In standard texts[1] it is stated that the glass transition temperature of orthophosphoric acid, H_3PO_4, is −121 °C. The source of the determination is not always cited but it seems to be that of Kobeko *et al.*[2] who determined T_g from measurements of the temperature dependence of the electrical conductivity of phosphoric acid. Although published almost 40 yr ago there does not seem to have been a more recent determination. We present here evidence from broad-line nuclear magnetic resonance (NMR) studies that the estimate of −121 °C for the glass transition temperature of orthophosphoric acid is wrong. A discussion of the interpretation of the electrical conductivity of phosphoric acid would be inappropriate beyond observing that several different ionic or molecular processes may be responsible for the transfer of electrical charges. Thus, an unambiguous determination of a glass transition from such data may be far from straightforward.

Broadline NMR spectra are obtained when solids or viscous liquids are studied, and the linewidth, δH, can be interpreted as a measure of the extent of molecular motion. With the onset of molecular motion the linewidth is reduced; this is because of the averaging effect of local molecular motion on the magnetic field 'seen' by the nuclei, such as the protons in the phosphoric acids.

In Fig. 1 typical proton NMR, ^1H, spectra are given for a sample prepared by dehydration at 300 °C of analytical reagent grade phosphoric acid, 85–90% H_3PO_4. Experimental methods used for the measurement of the NMR spectra are similar to those used for the determination of the T_g of α–D glucose penta-acetate and epoxy resins[3,4].

In experiments such as these the NMR linewidth is determined by the spin–spin relaxation time, T_2, and the linewidth, δH, is inversely related to this relaxation time

$$\delta H = \frac{\text{const}}{\gamma T_2}$$

where δH, the linewidth, is measured between the maxima and minima of the first derivative of the absorption signal (Fig. 1) γ is the gyromagnetic ratio and the value of the constant depends on the line shape[3,4].

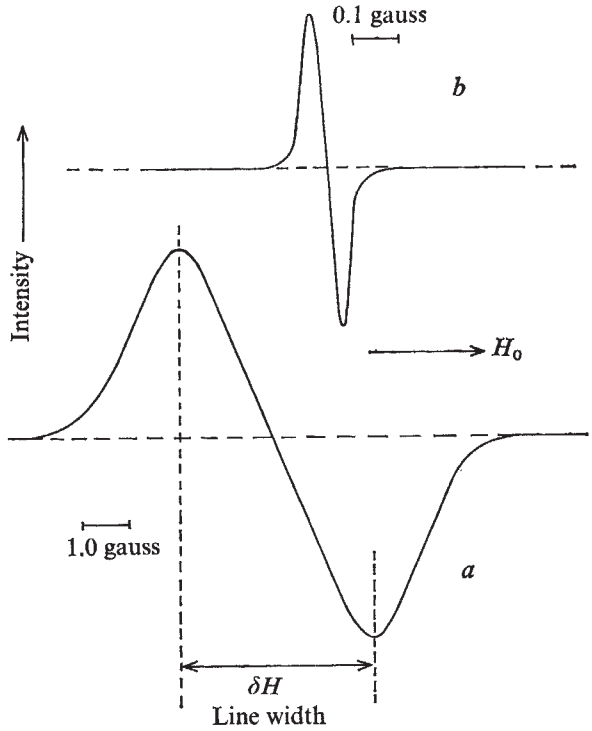


Fig. 1 First derivatives of NMR absorption spectra for phosphoric acid with $R = H_2O/P_2O_5 = 1.17$. *a*, Rigid glass at 169 K (−104 °C). *b*, Fluid liquid at 302 K (29 °C). The linewidth was determined from at least six repeat spectra. The spectrometer modulation was ~ 1/5 δH to avoid modulation broadening of the spectra. Measurements taken from ref. 6.

The change in linewidth with temperature for a phosphoric acid glass forming system is given in Fig. 2. At low temperatures the linewidth is large because of dipolar broadening because of the presence of other nuclei with magnetic moments. Thus, the linewidth is essentially determined by the local magnetic fields, H_{loc}, which depend on the local arrangements of neighbouring protons. When the temperature is raised sufficiently to permit molecular motion, the time average local magnetic field, $\langle H_{loc} \rangle$, is reduced. From NMR theory it is shown that relaxation processes have longer T_2 relaxation times when molecular motion occurs, and hence the linewidth δH decreases when T_2 increases.

Several methods have been proposed for the determination of glass transition temperatures from the change of linewidth with temperature[8], all of which yield essentially the same estimate of T_g when the transition is 'sharp'. The inflection point of the δH against T curve (Fig. 2) was used to estimate T_g for these phosphoric acid glasses. The transition illustrated in Fig. 2 is very sharp.

For specification of glass transition temperatures it is essential to include the experimental procedure in the operational definition of T_g, as was pointed out by Gee ~ 10 yr ago[5]. Maklakov and Pimenov[8] have discussed the relationships between NMR line narrowing and other methods of estimating T_g and McCall[9] has commented on NMR measurements of relaxation behaviour and the theories of glass transition behaviour. For the present purposes the inflection point

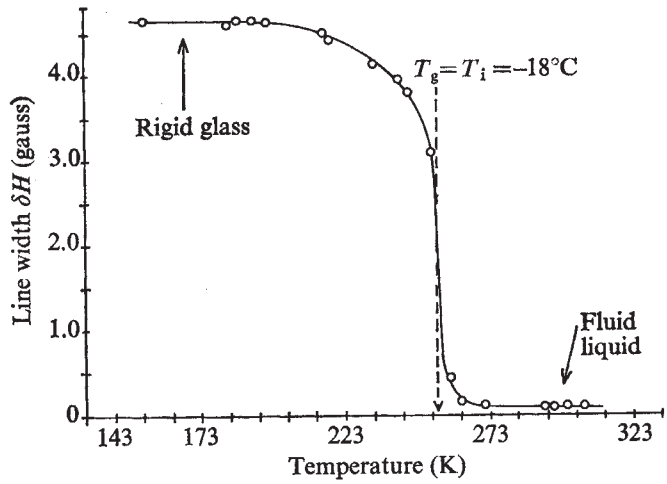
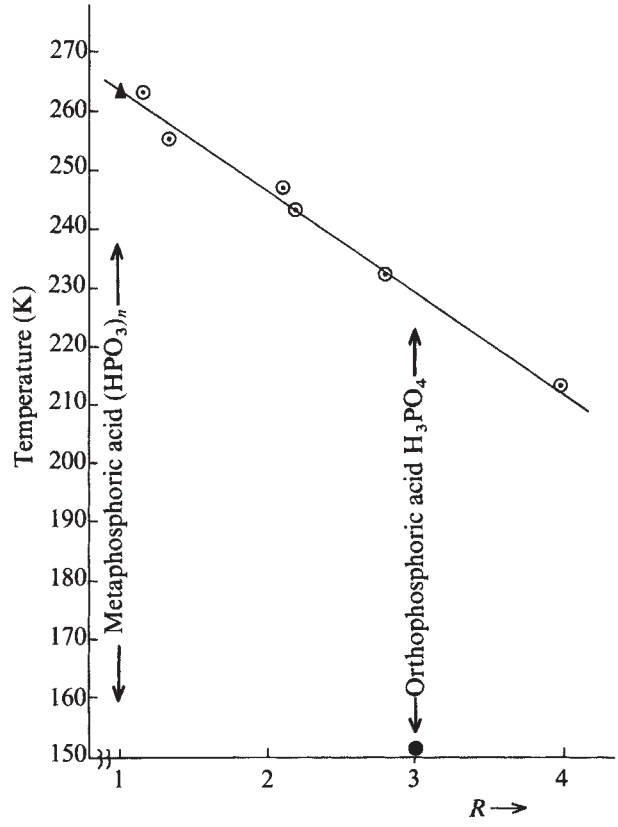


Fig. 2 NMR linewidth against temperature for phosphoric acid with $R = 1.33$. The glass transition temperature T_g is determined from the inflection point in the transition region[6].

of the NMR linewidth against temperature curve will be defined as the glass transition temperature, that is, $T_i = T_g$.

There are several theoretical empirical mixture rules for the correlation of the glass transition temperatures of two component systems[10], but none are established unequivocally. Thus, to represent the effect of composition of phosphoric acids on their glass transition temperatures it is convenient to plot T_g against R, where R is the molar ratio of H_2O/P_2O_4. Use of this compositional variable allows inclusion of metaphosphoric acid HPO_3, $R = 1$, and normal laboratory reagent 85–90% orthophosphoric acid, $R \simeq 4$. From Fig. 3, it is clear that there

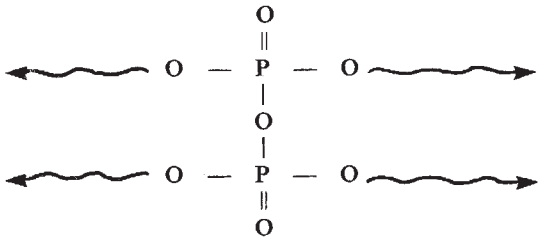
Fig. 3 Glass transition temperatures against R. T_g was determined from NMR linewidth against temperature graphs as in Fig. 2. The straight line was fitted by a linear regression procedure with the assumption that R was error free and all the error is in T_g, its equation is $T_g = 281.1 - 17.28\,R$, $1 \leqslant R \leqslant 4$. The correlation coefficient is 0.99_3. Data from ref. 6, $R = 1.17$ and 1.33; ref. 7, $R = 2.10, 2.78$ and 3.95; $R = 2.18$ (unpublished data of B.E. and N. A. Miller), ▲., ref. 11; ●, ref. 2.



is a linear relationship between T_g and R, when T_g is determined from the inflection point of the proton NMR linewidth temperature curve.

For the metaphosphoric acid, HPO_3 with $R = 1$, T_g has been determined by Eisenberg[11] using a linear variable differential transformer to measure the expansion of the sample with increase of temperature. There is agreement within 1 K between Eisenberg's determination of 263 K and that calculated from the equation given in Fig. 3 with $R = 1$, even though different routes were used for the preparation of the phosphoric acid samples. In spite of the complicated crystallisation behaviour reported for the metaphosphoric acids subject to different thermal treatments[1] it is clear that either dehydration of phosphoric acid at 300 °C or equilibration of a mixture of phosphorus pentoxide with water, yield products with the same glass transition temperature.

Caution is required in the application of the equation (Fig. 3) and it should not be used for values of $R < 1$. There is increasing cross linking as R is reduced below 1, because of the formation of trifunctional units through the elimination of water by condensation of hydroxyl groups on adjacent chains.



Glass transition temperatures are increased by cross linking but at present it is difficult to test relationships between glass transition temperatures and degree of cross linking. This is because reliable methods for the determination of the concentration of cross links may not be available[4]. Hence because of this increase in cross linking the use of the equation in the region $0 < R < 1$ is inappropriate. For compositions with $R > 4$, experimental determinations of T_g are required before it is possible to check on the applicability of this equation.

When a proton NMR spectrum has a narrow linewidth, $\delta H < 0.25$ gauss, there must be isotropic reorientation of the nuclei at a rate $> 10^4$ Hz, so that $\langle H_{loc} \rangle$ is effectively zero, and the measured line width is determined by the homogeneity of the applied magnetic field and other instrumental factors. When the signal is broad, $\delta H \sim 4$–5 gauss or more, then the protons are in relatively fixed positions. Thus, if a sample has a broad line it must be a solid. For these phosphoric acids the linewidth is broad, up to 5 gauss, even for the sample with $R \sim 4$ at $T < 200$ K. Thus this sample is a glass at temperatures < 200 K. While it is possible for an amorphous material to have molecular motion of various types at $T < T_g$, it is not possible for a sample to have a broad NMR line at $T > T_g$. Therefore, T_g for all these samples must be > 200 K. Kobeko's estimate of 152 K for the glass transition temperature of orthophosphoric acid is far too low.

The use of the definition $T_g = T_i$ (Fig. 2) together with application of our equation, allows estimation of glass transition temperatures in the composition range $1 \leqslant R \leqslant 4$. For orthophosphoric acid, $R = 3$, the predicted glass transition temperature is 229 ± 2 K.

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Regenerative failure of double half limbs in *Notophthalmus viridescens*

IT HAS been proposed that positional information in epimorphic fields is specified in terms of polar coordinates[1], where one component of positional information is a value corresponding to position on a circle, and the other to position on a radius. In the newt limb, the circular sequence of positional values lies around the circumference and the radial sequence lies in the proximal–distal axis of the limb. French *et al.* have proposed that various different regulative phenomena in such epimorphic fields as insect legs, insect imaginal disks, and amphibian limbs can be accounted for by two principles of cellular behaviour. The first of these is shortest intercalation, whereby any discontinuities in positional information are resolved by intercalation of missing positional values during growth. The second principle is that distal transformation can only take place from a complete set of positional values in the circular sequence. Unless a complete circle is either present at a site of amputation, or can be generated by intercalation from an incomplete circle, no regeneration (distal transformation) will occur. These principles have been used to account for the number, location, handedness and orientation of supernumerary regenerates produced after grafting experiments in amphibians[2] and insects[3]. The results reported here directly support the idea that a complete circle of positional values in the circular sequence is necessary for limb regeneration in adult newts.

Double half limbs were made in *Notophthalmus viridescens* as shown in Fig. 1. Controls were sham operated limbs in which either an anterior or a posterior half of an upper arm had been removed and replaced in normal orientation. All grafts healed into place rapidly and became fully vascularised by an average of 14 d after operation. The regenerative ability of the controls and the double half limbs was tested by amputating through the grafted region, close to its distal end, usually after 3–4 weeks, but sometimes after 8–9 weeks (eight double half limbs).

All 18 control limbs regenerated normally (Table 1 and Fig 2*e*), showing that the techniques used do not impair regenerative ability, and that an interval of 3–4 weeks between grafting and amputation is sufficient for restoration of an adequate nerve supply at the amputation plane. When double half limbs were amputated, however, regeneration either failed or was defective. The results were similar for both double anterior and double posterior limbs, and for limbs which were amputated 3–4 weeks and 8–9 weeks after grafting. Of 16 double anterior limbs tested (Table 1), none regenerated normally, and of 18 double posterior limbs,

Table 1 Regenerative ability

Limb type	Total	Defective or no regeneration	Normal regeneration
Control posterior	11	0	11
Control anterior	9	0	9
Double posterior	18	17	1
Double anterior	16	16	0
Half posterior	32	5	27
Half anterior	30	4	26

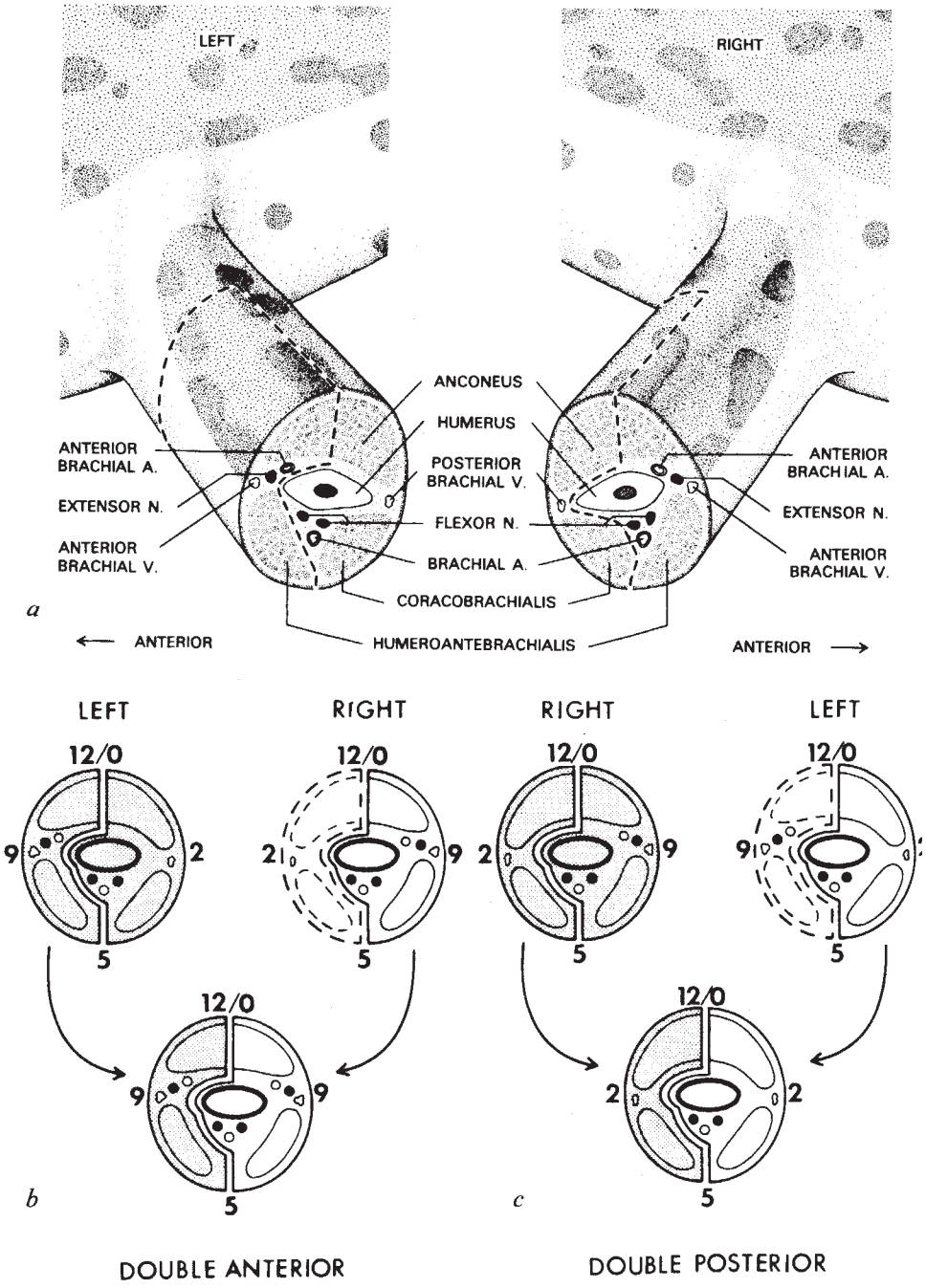

Fig. 1 Diagram of the grafting operation. *a*, Left and right limbs, cut away distally, to show the major anatomical features. The major muscles of the upper arm are the anconeus, coracobrachialis and humeroantebrachialis. A, Artery; N, nerve; V, vein. To make a double anterior limb, a 1.5-mm long section of the posterior half of a right upper arm was removed by making mid-dorsal and mid-ventral incisions, as well as proximal and distal incisions in the posterior half of the limb, down to the bone. The loosened posterior half was pulled away and discarded. An anterior half limb of matching dimensions from the left upper arm was grafted on to the right limb in place of the discarded posterior half. The graft was oriented normally with respect to its dorso–ventral and proximal–distal axes. Double posterior limbs were made by grafting the posterior half of the right upper arm in place of the anterior half of the left. *b* and *c*, Schematic representations of the operations to form double anterior and posterior limbs. Key positional values in the circular sequence are indicated by numbers from 0 to 12. Operations were performed using chloretone anaesthesia. In all cases, the mid-ventral nerves and artery were left undisturbed on the limb stump. All grafts were stained for 2 min in a 0.05% solution of methylene blue in Holtfreter solution[11] before grafting. Grafts were held in place with small pieces of damp lens paper, and the operated animals were kept cold (10 °C) for 2 d. Between the time that the animals were removed from the cold and the time that their limbs were amputated, they were kept at room temperature (20 °C). Thereafter they were maintained at 25 °C for the remainder of the experiment (2–2.5 months).

only one formed a normal distal regenerate. The range of skeletal structures formed by the double half limbs 2–2.5 months after amputation varied from little more than a cartilaginous cap on the cut end of the humerus (Fig. 2*c*) or a small separate cartilaginous nodule (Fig. 2*b*) to several small skeletal elements arranged in series (Fig. 2*a* and *d*). These results provide support for the hypothesis of French *et al.*[1] that a complete set of positional values in the circular sequence is necessary for distal transformation in amphibian limbs. The results of preliminary experiments performed on *Ambystoma tigrinum* by Stocum (personal communication) confirm this main result in another species of amphibian.

Previous experiments[4,5] provide further support for the complete circle rule in amphibian limbs. Limbs rendered incapable of regeneration by X irradiation could be caused to regenerate if they were provided with a normally oriented cuff of non-irradiated skin, that is, one containing a complete set of positional values in the circular sequence. It was also shown, however, that a cuff of skin which was oriented so that only a single 'quality' (that is dorsal, ventral, anterior or posterior) was present at the amputation site could not cause X-irradiated limbs to regenerate. Although the amount of non-irradiated tissue available for regeneration was the same in both instances, regeneration occurred only when this tissue was oriented so as to produce a complete set of positional values in the circular sequence at the amputation site.

Further evidence for the relationship between a complete circle and normal distal transformation comes from the lateral supernumerary regenerates which sometimes formed in the grafting experiments (Fig. 2*d*). In 10 of 18 double posterior limbs and in one of the 16 double anterior limbs (but never in the control limbs), regenerative changes leading to the formation of a complete lateral supernumerary limb were initiated at the proximal graft–host junction when the limb was amputated through the distal region of the graft. A complete, lateral circle of positional values is

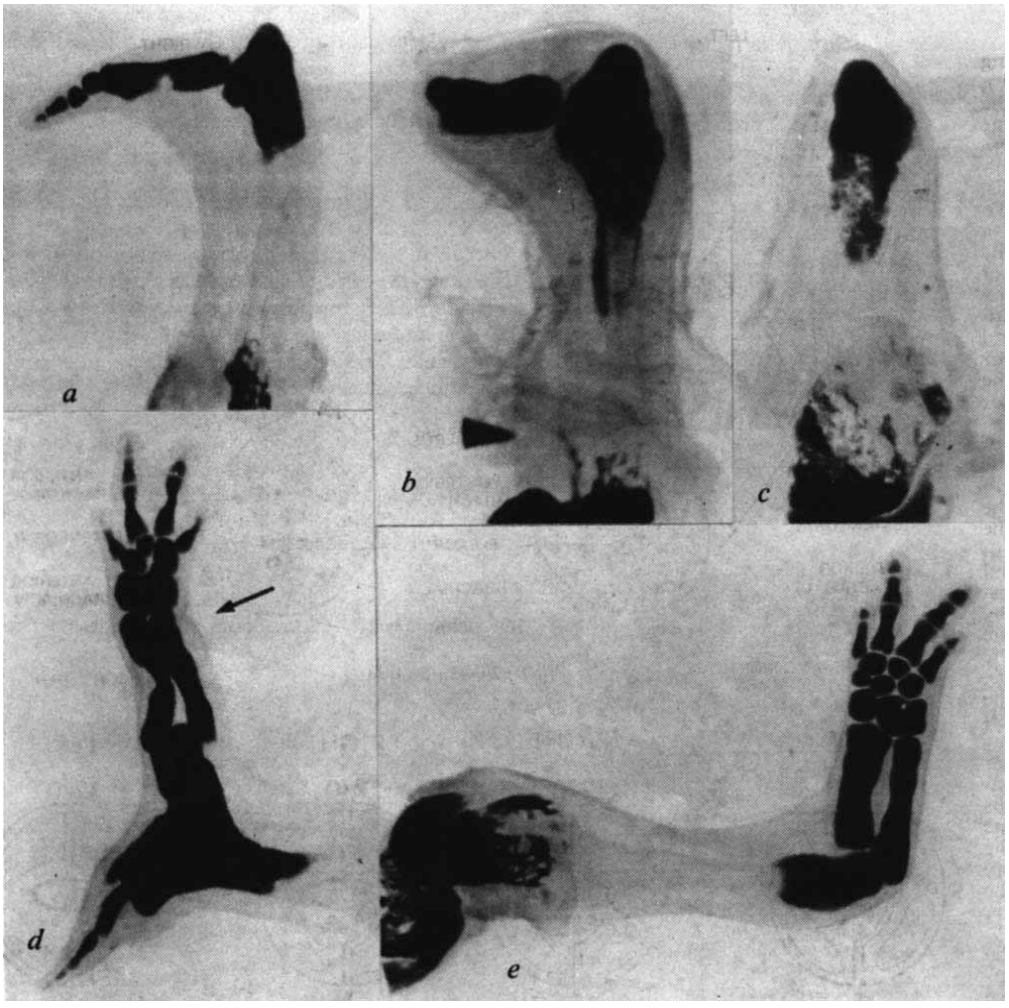

Fig. 2 Skeletal preparations of control and experimental limbs, prepared by the Victoria blue technique[12] 2–2.5 months after amputation. *a* and *b*, Double anterior limbs. *c* and *d*, Double posterior limbs, showing the spectrum of structures produced at the distal amputation site. In (*d*) a lateral supernumerary regenerate (arrow) has developed from the proximal graft–host junction. *e*, A control limb in which the posterior half of the upper arm had been removed and replaced.

created at this position in the experimental limbs (Fig. 3), with half of the values provided by the host and half by the graft. Preliminary evidence from similar grafts made in axolotls indicates that such supernumerary limbs contain cells of both host and graft origin (my unpublished results with Krasner).

The details of the mechanisms involved in distal transformation, and the need for a complete set of values in the circular sequence are unknown. In spite of their failure to complete distal transformation, in these experiments all double half limbs initiated regeneration. Except for one case, however, none proceeded beyond the stage of late bud[6] and the majority were arrested at earlier stages.

A possible alternative interpretation of the failure of double half limbs to regenerate normally is that they have an insufficient number of nerve fibres at the site of amputation. This explanation is untenable for several reasons. First, the major mid-ventral limb nerves were left intact in all cases (Fig. 1). Second, control limbs always regenerated normally. Third, doubling the interval between grafting and amputation from 3–4 weeks to 8–9 weeks, to allow even longer for innervation to occur, did not alter the regenerative performance of double half limbs. Finally, more evidence comes from a multiple grafting experiment in which a double anterior limb is made, left to become vascularised and innervated, then the original host anterior half is replaced by a grafted posterior half, to form a reversed but normal limb. When such limbs are amputated after an appropriate interval, regeneration of a disharmonic limb occurs as expected from the reversed but complete limb stump (my unpublished results). These preliminary results, in addition to the other evidence cited here, indicate that the ability of double half limbs to become adequately innervated is not impaired, and that this alternative interpretation of the failure of double half limbs to regenerate can be rejected.

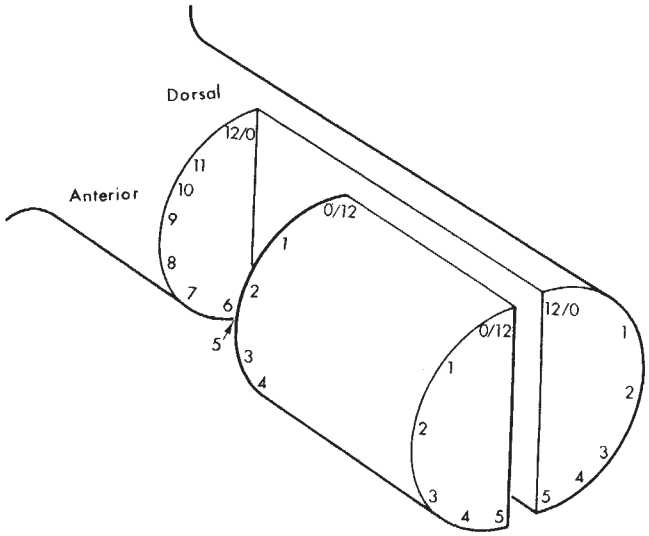

Fig. 3 Diagram of a double posterior limb stump indicating the complete circle of positional values at the proximal graft–host junction. The placement of positional values around the circumference is slightly non-uniform, as suggested by previous results and discussed in ref. 2. The graft is shown to be shorter than it actually is in order that the proximal graft–host junction can be clearly visualised.

In these experiments the regenerative ability of half limbs was also examined (Table 1). In each animal in which one double half limb was produced, the limb from which the graft was taken remained as a half limb, either half anterior or half posterior. These half limbs, as well as half limbs produced on both forelimbs of the same animal were either amputated immediately through the most distal portion of the half limb, or amputated 3–4 weeks later. Most of these limbs developed complete and normal regenerates, thus confirming earlier findings that half limbs can regenerate normally[7,8] or relatively normally[9]. The initial outgrowth did not arise at the most distal cut end of the half limb, but was eccentrically located towards the distal end of the lateral wound edge. During the later stages of regeneration, the regenerate became more symmetrically disposed until by the stage of late digits, little or no asymmetry could be detected. A tentative interpretation of this finding is that the eccentric location of the initial outgrowth is the position on the wound surface at which cells comprising a complete circle of positional values come together during wound healing from the proximal and distal half limb stumps. This possibility is being investigated.

The many similarities between the regulative behaviour of insect and amphibian limbs have led to the hypothesis that the mechanisms of pattern regulation in these distantly related organisms are the same[1]. This idea is strengthened by the similarity of the results reported here to those reported by Bohn[10] with double dorsal and double ventral cockroach legs.

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Detection and repair of single-strand breaks in nuclear DNA

DIRECTLY or indirectly phosphodiester bonds in DNA are broken when living cells are irradiated by ionising radiations or ultraviolet light. There are various sophisticated techniques for monitoring radiation damage[1–6]. We describe here how radiation damage in DNA and its repair can be detected simply in the white cells of human blood. The method is very sensitive and should prove useful in screening populations for abnormal repair mechanisms. As there is also great interest in methods for detecting environmental agents that damage DNA[2,7], we have applied the method to detect the damage caused by mitomycin C.

We have described our technique for detecting the damage caused to the nuclear DNA of HeLa cells by very low doses of γ rays and also for monitoring the repair of this damage[8,9]. When cells are lysed in the presence of non-ionic detergents and high salt concentrations, structures resembling nuclei—called nucleoids—are released. These nucleoids contain nearly all nuclear RNA and DNA, but are depleted of nuclear proteins[10]. Their DNA is supercoiled and compact so that the nucleoids sediment more rapidly in sucrose gradients than their γ-irradiated counterparts which contain extended DNA with single-strand breaks or nicks. Repair of the breaks restores the DNA to its original conformation and re-establishes the normal sedimentation rate. We monitor DNA integrity by measuring the distance sedimented by nucleoids in sucrose gradients.

Nucleoids are released immediately on addition of white blood cells to a lysis mixture containing the non-ionic detergent Triton X-100 and 1.95 M NaCl. The effects of the intercalating dye, ethidium bromide (EB), on the sedimentation of nucleoids derived from white blood cells was studied by spinning the nucleoids through sucrose gradients containing 1.95 M NaCl and different concentrations of the dye (Fig. 1). The distance travelled by the nucleoids is expressed as a ratio relative to that travelled by unirradiated nucleoids sedimenting in the same conditions but in the

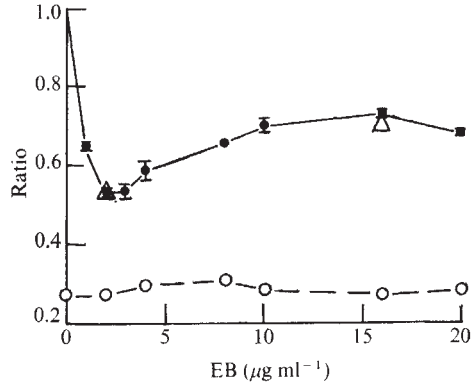

Fig. 1 Effect of EB on the sedimentation of nucleoids from human white blood cells. The distance sedimented by nucleoids in gradients containing different concentrations of EB is expressed as a ratio relative to that of unirradiated nucleoids sedimenting in the absence of EB. ●, Unirradiated nucleoids; ○, nucleoids γ irradiated (960 rad, 120 rad min^{-1}) after addition of cells to the lysis mixture[8]; △, unirradiated nucleoids from a patient with xeroderma pigmentosum. Error bars give the standard error of the mean. Human white cells were obtained from peripheral blood[17] and cultured in RPMI 1640 medium supplemented with 20% foetal calf serum (Flow Laboratories). Between 1 and 5% of the cells prepared in this way are red cells, the remainder are almost all lymphocytes. The results obtained with blood from four normal males and two females were pooled. In some cases white cells stored in medium on ice for 24 h were used; the sedimentation properties of nucleoids prepared from such cells were similar to those obtained from freshly isolated cells. Samples of 50 μl of a suspension containing between 2 and 5×10^5 cells in phosphate-buffered saline (PBS) were layered on 150 μl of a lysis mixture floating on top of "isokinetic" sucrose gradients[8,10]. Gradients (15–30% sucrose, 4.6 ml, *p*H 8.0) contained 1.95 M sodium chloride, 0.01 M Tris, 0.001 M EDTA in addition to various concentrations of EB. The lysis mixture contained sodium chloride, EDTA, Tris and Triton X-100 in amounts which, on addition of 1 volume of PBS containing cells to 3 volumes of the mixture, gave final concentrations of the constituents of 1.95 M, 0.1 M, 2 mM and 0.5% respectively. (For the purpose of calculating the final concentration of sodium chloride, the contribution of the PBS is neglected). Fifteen minutes after the addition of the cells to the lysis mixture, gradients were spun at 30,000 r.p.m. for 25 min at 20 °C in the SW50.1 rotor in a Beckman L2-65b ultracentrifuge. After the gradients had been spun, the position of the nucleoids in the gradient was determined by their absorbance at 254 nm. One gradient of the six spun in the rotor served as a reference; the distance travelled by nucleoids in other tubes is expressed relative to the distance sedimented by nucleoids in the reference tube[8].

absence of EB. As the concentration of EB in the gradient is increased, the distance travelled by nucleoids falls to a minimum and then rises again. γ Irradiation reduces the sedimentation rate of the nucleoids and abolishes the biphasic response to EB. The shape of a similar curve obtained with HeLa nucleoids is discussed elsewhere[8]. As the sedimentation rate of supercoiled DNA varies in this characteristic biphasic manner[11], we conclude that nucleoid DNA is supercoiled. Irradiation introduces single-strand breaks into the DNA so that supercoils are lost.

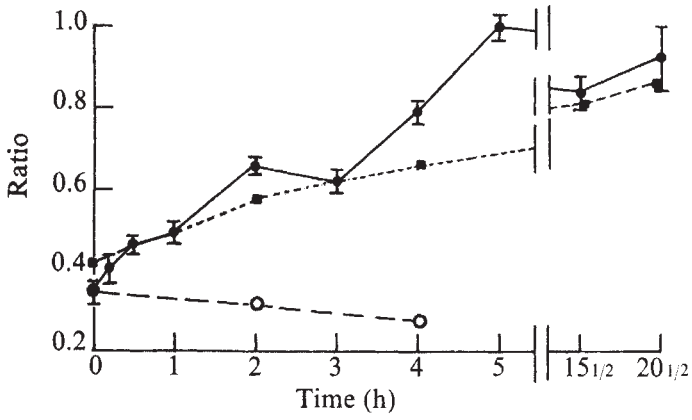

Fig. 2 Effect of incubation of white blood cells after γ irradiation on nucleoid sedimentation. The distance sedimented by nucleoids from irradiated cells in gradients lacking EB is expressed as a ratio relative to that of nucleoids from unirradiated cells which had been treated similarly. White cells (2×10^6 per ml in medium) were irradiated (960 rad)[8], and incubated for different times at 37 or 4 °C. They were then added to 10 volumes of ice-cold PBS, pelleted and resuspended in ice-cold PBS. Samples of 50 μl were applied to gradients which were then spun and analysed as described in the legend to Fig. 1. Error bars give the standard error of the mean. Normal white blood cells, irradiated and incubated at 37 °C (●) or 4 °C (○). White cells from a patient with xeroderma pigmentosum, irradiated and incubated at 37 °C (■).

Repair of the damage caused by γ irradiation was studied by irradiating white blood cells (960 rad) and then incubating them for different periods before applying them to gradients lacking EB and then measuring the sedimentation rate of the nucleoids. (A dose of 960 rad reduces the cloning efficiency of HeLa cells by 99% (ref. 8.) Incubation at 37 °C after irradiation, but not at 4 °C, increased the sedimentation rate of the nucleoids (Fig. 2). After 5 h, the sedimentation rate had been restored almost to that of unirradiated nucleoids, and the nucleoids from the irradiated cells had regained their biphasic response to EB (Table 1).

These techniques have been extended to include measurements on the effects of ultraviolet light. Repair of damage induced by ultraviolet light involves cutting one strand of the DNA duplex ("incision"), removal of the principal photoproduct, the thymine dimer ("excision"), synthesis of DNA complementary to the unaffected strand and "ligation" of the final phosphodiester bond to restore the intact duplex[5]. HeLa cells were irradiated (25 erg mm^{-2}) and incubated for different times at 37 °C before the sedimentation rate of nucleoids isolated from them was measured in gradients lacking EB (Fig. 3*a*). (A dose of 25 erg mm^{-2} reduces the cloning efficiency of HeLa cells by 24% (our unpublished results).) Irradiation without subsequent incubation at 37 °C reduces the rate of sedimentation. This probably results from the single-strand breaks induced by the highly active incision enzymes of HeLa cells during the time taken to concentrate the cells before they are lysed. (Although the formation of a thymine dimer in DNA unwinds the double helix by 5–6° (ref. 12), the sedimentation rate is unlikely to be reduced by such unwinding in the absence of strand scission because irradiation of isolated nucleoids with high doses of ultraviolet light does not alter the sedimentation rate (our unpublished results).) Incubation of the cells at 37 °C for 5 min after irradiation reduced the rate of sedimentation of the nucleoids further, whereas incubation periods greater than 20 min increased the distance sedimented until it became indistinguishable from that of the unirradiated controls. We conclude that after irradiation, incision initially leads to a partial loss of supercoils in nucleoid DNA, but then ligation restores supercoiling (Fig. 3*a*).

A measure of the damage caused by different doses of ultraviolet light is given by the distance sedimented in the absence of EB by nucleoids after the irradiated cells had been incubated for 5 min at 37 °C to maximise the number of strand breaks (Fig. 4). As radiation dose increased, the distance sedimented decreased progressively.

The damage caused by ultraviolet light is repaired much more slowly in white blood cells than in HeLa cells. Strand breakage and loss of supercoiling were maximal 2 h after irradiation. The normal supercoiled configuration was not fully recovered after 15.5 h (Fig. 3*b* and Table 1). There was

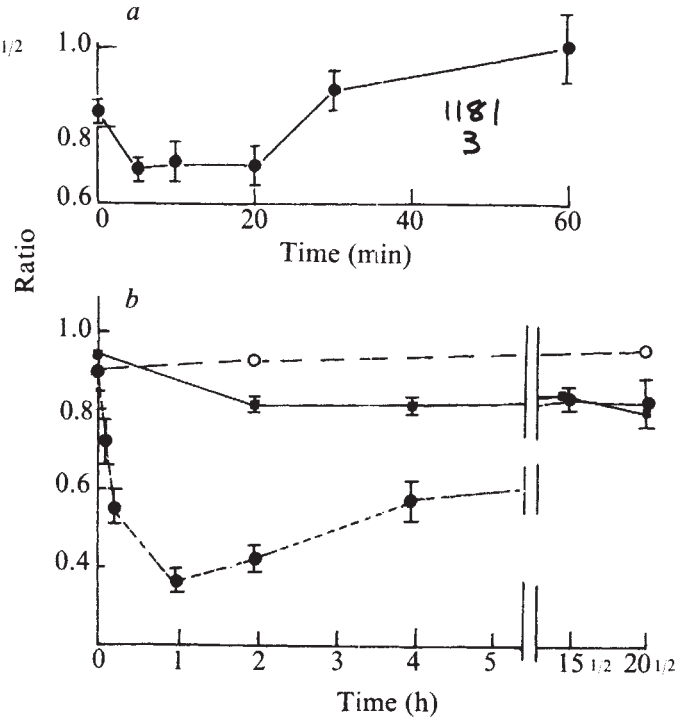

Fig. 3 Effect on nucleoid sedimentation of incubating (*a*) HeLa cells and (*b*) human white blood cells at 37 °C after irradiation with ultraviolet light. *a*, Human HeLa cells were grown in suspension[8]. 0.5 ml of cells (2×10^6 per ml) in ice-cold medium in a Petri dish were irradiated (25 erg mm^{-2}, 5 erg mm^{-2} s^{-1}) with ultraviolet light while the Petri dish was shaken vigorously. The source of ultraviolet light was a Sylvania germicidal tube (G 15T8). Doses were measured with an ultraviolet meter[18]. After irradiation, cells were mixed with 4.5 ml of medium at 37 °C and incubated in the dark at 37 °C. At the end of incubation the cells were mixed with 9 volumes of ice-cold PBS, pelleted and resuspended in ice-cold PBS. Samples of 50 μl of the cell suspension were applied to sucrose gradients lacking EB. Gradients were spun at 5,000 r.p.m. for 50 min and analysed as described in the legend to Fig. 1. The distance sedimented by nucleoids derived from irradiated cells is expressed as a ratio relative to the distance sedimented by nucleoids derived from cells treated similarly but which had not been irradiated. Error bars give the standard error of the mean. *b*, The experiments with white blood cells were similar to those with HeLa cells except that media and spinning conditions were different (see legend to Fig. 1). ●, Normal white blood cells, irradiated and incubated at 37 °C; ○, normal white blood cells, irradiated and incubated at 4 °C; ■, white blood cells from a patient with xeroderma pigmentosum, irradiated and incubated at 37 °C.

Table 1 Effect of different treatments on supercoiling in the nucleoids from white blood cells

Treatment	Relative distance sedimented by nucleoids in the presence of EB			
	0 μg ml^{-1}	2 μg ml^{-1}	4 μg ml^{-1}	16 μg ml^{-1}
Untreated	1.0	0.53	0.59	0.73
4-h incubation at 37 °C after γ irradiation (960 rad)	0.82	0.53	0.66	0.65
2-h incubation at 37 °C after ultraviolet irradiation (25 erg mm^{-2})	0.39	0.39	—	0.35
15.5-h incubation at 37 °C after ultraviolet irradiation (25 erg mm^{-2})	0.84	0.58	—	0.68

Nucleoids obtained from cells treated in different ways were spun in gradients containing different concentrations of EB. The distance sedimented by nucleoids from treated cells is expressed as a ratio relative to that of nucleoids from untreated cells sedimenting in the absence of ethidium. The conditions used for the untreated cells, and cells irradiated with γ rays or ultraviolet light are described in the legends to Figs 1, 2 and 3, respectively.

little change in the sedimentation rate of nucleoids when the irradiated cells were incubated at 4 °C (Fig. 3*b*).

Cells from patients with the autosomal recessive disease, xeroderma pigmentosum can repair the damage caused by ionising radiation but cannot effectively repair DNA damaged by ultraviolet light. Some forms of this disease are thought to be characterised by deficiency in "incision" activity[5]. Nucleoids prepared from the white cells of our patient sedimented in gradients containing EB exactly as the nucleoids from normal cells (Fig. 1). In the absence of EB the sedimentation behaviour of nucleoids prepared from γ-irradiated cells was also indistinguishable from that of nucleoids prepared from normal cells that had been irradiated (Fig. 2): the patient's cells could thus repair γ-ray damage. On the other hand, irradiation with ultraviolet light affected normal cells and those of the patient differently (Fig. 3*b*). When the patient's cells were irradiated with ultraviolet light and incubated at 37 °C the sedimentation rate of the nucleoids in the absence of EB was not reduced as much as that of controls : the patient's cells were unable to "incise" normally and the nucleoids retained supercoils and sedimented rapidly.

Mitomycin C is a carcinogen[7]. After activation by cellular enzymes it cross links DNA strands without breaking them. Strands are broken only when the damaged region is removed by repair enzymes[13]. We investigated the effects

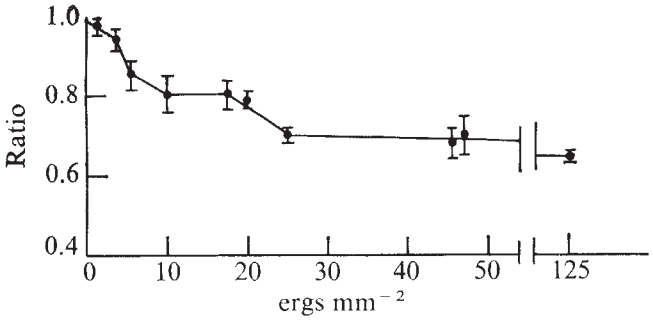

Fig. 4 Effect of different doses of ultraviolet radiation on the sedimentation of HeLa nucleoids. HeLa cells were irradiated for 5 s with different doses of ultraviolet light, incubated for 5 min at 37 °C, applied to sucrose gradients lacking EB and the gradients were spun and analysed (see legend to Fig. 3). The distance sedimented by nucleoids derived from irradiated cells is expressed as a ratio relative to the distance sedimented by nucleoids derived from cells which had been treated similarly but which had not been irradiated. Error bars, s.e.m.

of mitomycin C on the integrity of DNA by incubating white cells at 37 °C for 2 h in different concentrations of the drug, lysing the cells, and determining the rate of sedimentation of the nucleoids in the absence of EB (Fig. 5). Low concentrations of the drug had no effect on the distance sedimented, higher concentrations reduced it. At the high concentrations, strand breakage caused loss of supercoiling and slow sedimentation. The effects of lower concentrations can be detected by incubating the cells with the drug for longer periods (our unpublished results). If, after a 2-h exposure to the drug, the cells were washed and incubated in its absence at 37 °C for 15.5 h, faster sedimentation was restored, repair of the damage restored normal supercoiling.

We have made nucleoids containing superhelical DNA from a wide range of cells (fibroblasts, lymphoblasts, erythroblasts, teratocarcinoma and epithelial cells of men, mice, birds, amphibians and insects (ref. 9 and our unpublished observations)), so that these methods for detecting single-strand breaks in DNA should be applicable to most cells.

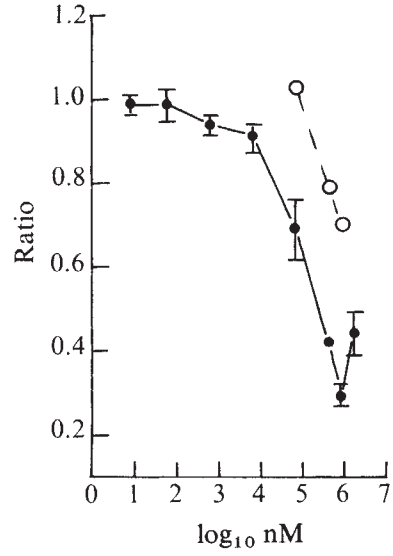

Fig. 5 Effect of mitomycin C treatment of white cells on the sedimentation of nucleoids in gradients lacking EB. White cells were incubated for 2 h at 37 °C (see legend to Fig. 1) in the presence of different concentrations of mitomycin C (Boehringer). Some cells were then collected and applied to gradients immediately and the gradients were spun and analysed (see legend to Fig. 1). Other cells were washed twice with medium, resuspended at 2×10^6 per ml in medium lacking mitomycin C and incubated a further 15.5 h at 37 °C. They were then collected and treated as the others.. The distance sedimented by nucleoids derived from cells treated with mitomycin C is expressed as a ratio relative to the distance sedimented by those derived from untreated cells. Error bars, s.e.m. ●, Cells incubated for 2 h in the presence of mitomycin C. ○, Cells incubated for 2 h in the presence of mitomycin C, washed and then incubated for a further 15.5 h at 37 °C.

Using white cells obtained from human blood taken in the morning, we can assess by the evening whether repair of damaged DNA in the cells is normal. While doses of 960 rad (γ rays) or 25 erg mm^{-2} (ultraviolet light) produce the large effects described in these experiments, much lower doses (that is, 12 rad and 5 erg mm^{-2}) can be detected. The method should therefore be particularly well suited to the rapid screening of patients for deficiencies in the mechanisms that repair radiation damage.

Many carcinogens and mutagens break phosphodiester bonds in cellular DNA either directly or indirectly after enzyme action. Breakage, which can be demonstrated by

conventional physical techniques[14] probably increases repair synthesis[15] and recombination[2,16]. Agents that break DNA can be detected in nucleoids with the sensitivity, rapidity and economy usually associated with the use of bacteria[7]. There may well be advantages in using human cells in any screening method for detecting potential human carcinogens.

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Evidence of a thymic abnormality in murine muscular dystrophy

The involvement of the thymus in muscular, neuromuscular and neurological disorders is well documented[1-4]. In spite of the extensive study made of the thymus in myasthenia gravis, which, as a neuromuscular disorder with clinical symptoms of muscular weakness, shares some similarities with muscular dystrophy, the state and function of the thymus in muscular dystrophy has been ignored. The poor health and susceptibility to infection of dystrophic mice have frequently been observed. These observations and the similarities between dystrophic wasting and that seen in mice thymectomised pre- or neonatally[5], led us to the conclusion that an investigation into the status of the thymus in murine muscular dystrophy was warranted. We have found that the thymuses of dystrophic mice exhibit changes in the age-dependent variation in thymic weight, together with aberrations in cellular morphology indicative of altered secretory activity.

Male and female dystrophic mice of the Bar Harbor 129/ReJ strain were killed by cervical dislocation at various ages, their thymuses, spleens and elbow lymph nodes removed, dissected free of fat and connective tissue, weighed and fixed in 10% formol-saline or methanol. The thymuses, spleens and lymph nodes from clinically normal littermates, and also from a non-dystrophic strain of mice (CBA), were used for comparison. The organs were paraffin embedded and subjected to routine haematoxylin–eosin staining procedures. Unna–Pappenheim staining was also used[6]. The histology of random single sections of these organs was then compared.

For an initial indication of thymic status, the thymus weights of normal and dystrophic mice were compared. It is not possible to compare thymic indices in these animals because of the selective wasting of hind limb muscle in the dystrophic mouse. Generally, mouse thymuses have been found to decline in weight from 4 to 12 weeks of age; the thymuses of the normal littermates followed this trend. In dystrophic mice, the thymic weights were at first much lower, but then increased with age, reaching a maximum at approximately 9 weeks of age, at a weight comparable to that of the normal mouse at 4–6 weeks of age. The thymic weights of the dystrophic mice then slowly declined to a level approaching that of their normal littermates at 12 weeks of age (Fig. 1). In all strains of mice so far studied, there has been to our knowledge, no report of a similar weight gain to 9 weeks of age, the predominant pattern being an increase in weight to 4 weeks of age,

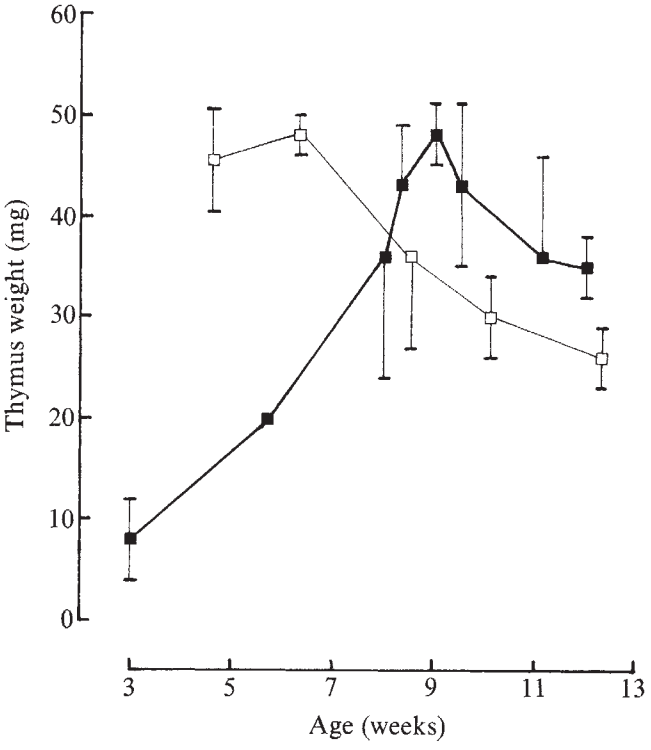

Fig. 1 Thymus weight as a function of age in normal and dystrophic mice. Thymus weight (mg)±s.d. Normal mice, 4–7 animals per group. Dystrophic mice, 2–6 animals per group.

followed by a gradual, but definite, decrease in weight which continues throughout the life span of the animal[7].

This anomaly led us to consider the histology of the thymus of dystrophic mice. As shown in Fig. 2, this also differed dramatically from the normal. While the normal littermates showed no abnormalities of basic thymic morphology, a number of the dystrophic animals studied exhibited remarkable alterations of thymic cellular composition. In the main, this consisted of a depletion, and in some cases, total absence of lymphocytes from lobules of the thymus, these being replaced by epithelial cells which showed very marked signs of secretory activity[8]. In extreme cases, the epithelial cells had formed themselves into classical, though duct-less acini, surrounding a material which did not stain in the conditions used. These drastic changes in thymic composition were found in young (3–8 weeks of age) dystrophic mice which were severely affected by the disease, as evidenced by the degree of disability. In older animals, and those less severely affected, the thymus showed less dramatic changes. These consisted of an apparent relative increase in medullary area over cortical area and a loss of distinction of the cortico–medullary junction. The change in the ratio of medulla to cortex could be due to an increase in medullary tissue or a decrease in the number of lymphocytes populating the

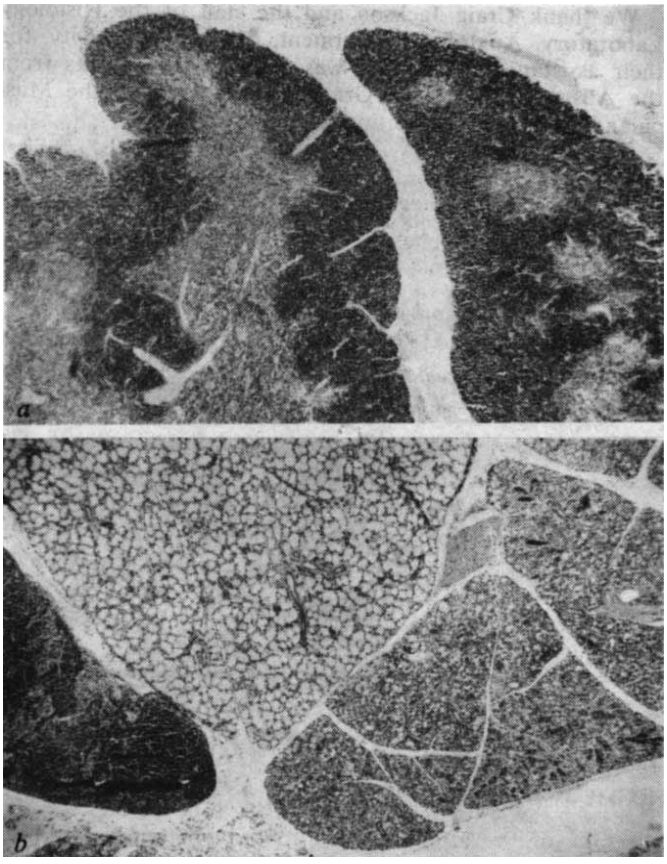

Fig. 2 Histology of thymuses from normal and dystrophic mice. *a*, Thymus from clinically normal littermate. The cortico–medullary junction is distinct; few epithelial cells can be detected in the cortex which is tightly packed with lymphocytes. Haemotoxylin and eosin, ×10.4. *b*, Thymus from severely affected dystrophic mouse. The three lobules shown were enclosed by a continuous capsule of thymic epithelium. The lobule on the left most closely resembles the normal state, but even here, patches of lighter staining cells can be seen in the outer cortex. The lobule on the right represents an intermediary stage in which the loss of definition of the cortico–medullary junction, the depletion of the lymphocytes present in the lobule, and the apparent increase in the number of epithelial cells, was the earliest and most consistent observation. The centre lobule shows the most extreme degree of alteration, having no resemblance to the normal thymus. The nuclei of what are presumed to be epithelial cells because of the presence of cytoplasm around the nuclei form a rim around an accumulation of poorly staining material, the chemical nature of which is unknown. Haemotoxylin and eosin, ×10.4.

cortex. As the packing of lymphocytes in the cortex was noticeably less dense, the latter explanation seems more probable. The cortico–medullary junction, which is normally quite distinct, was indefinite in dystrophic mice, the change from medulla to cortex taking place over a much larger area than is seen in the normal littermates. In some thymuses from dystrophic mice an infiltration of epithelial cells from the outer rim of the cortex, into the cortex, was also seen. The main changes seen in the thymuses in this study are summarised in Table 1.

As all these findings indicated a loss or lack of lymphocytes in the thymus of dystrophic mice, the spleen and lymph nodes were investigated as possible recruiting areas for the missing lymphocytes. Dystrophic spleen weights were found to differ from those of normal mice, the spleens of dystrophic mice being consistently smaller at all ages than those of their normal littermates (Fig. 3). It was also noted that while spleen weights of normal mice generally exhibited a certain variability, reflecting the ability of the individual animals to respond to infectious agents and other stimulants of the immune system (splenomegaly), the spleen weights of the dystrophic mice were relatively constant. Investigation of splenic histology did not reveal any gross differences between normal and dystrophic mice. It was, however, noted that no germinal centres, as detected by Unna–Pappenheim staining, were seen in the spleens of dystrophic mice, although they were often found in the spleens of normal animals. The lymph nodes of dystrophic animals seemed to contain fewer lymphocytes and relatively more endothelial cells than the lymph nodes of normal animals.

These observations and the peculiarities of thymic weight and thymic histology all indicate a possible defect in function of the immune system in dystrophic mice. The altered morphology of the thymuses of dystrophic mice indicate an abnormal secretory activity of the epithelial cells of the thymus. These cells have been implicated as the site of synthesis and secretion of thymus hormones in normal mice[8,9]. A wide variety of actions have been ascribed to thymic hormones; notably, these include both potentiating and inhibitory actions on all immune functions, the capability of interacting with a variety of other endocrine hormones, and hence actions on growth, muscle regeneration and the capacity of the animal to respond to disease[10]. If, as the evidence suggests, there is a defect somewhere in the system of thymic hormones and/or their actions, this

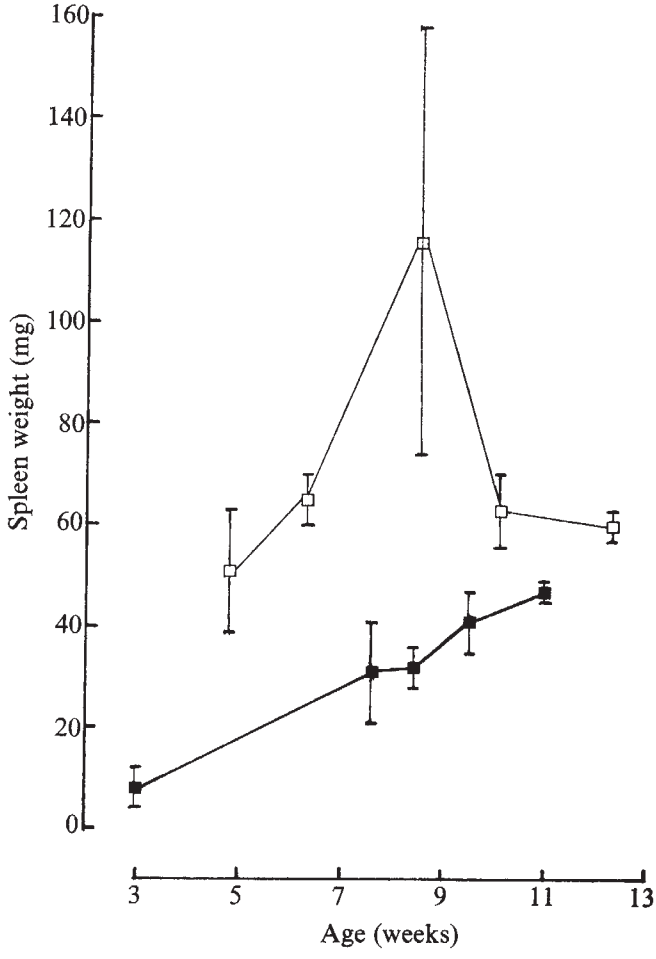

Fig. 3 Spleen weight as a function of age in normal and dystrophic mice. Spleen weight (mg)±s.d. Normal mice, 4–7 animals per group. Dystrophic mice, 4 animals per group. The large variation in spleen weight of normal mice observed at 8.5 weeks of age is interpreted as a reflection of the capacity of these mice to undergo splenomegaly (see text). □, normal; ■, dystrophic.

could explain a well known, but generally ignored, characteristic of murine muscular dystrophy. The disease has a random clinical onset and remarkably variable progression, some animals dying at 3 weeks of age, others surviving to 6 months. If this disease has an hormonal basis, then individual animals would be expected to have different capacities to compensate for an hormonal excess or insufficiency, inactivity or hyper-reactivity, by alteration of other hormones with which the affected hormone interacts. The intricacies of such interaction and compensation between endocrine hormones, and the variation between individuals has been reviewed[11]. Furthermore, the finding that the thymus weights of dystrophic mice follow an age-pattern different from that of normal mice, regardless of severity of disease, or extent of thymic abnormality, suggests that the thymus is abnormal in all dystrophic mice. The effect and extent of this abnormality may be defined by a more complex system, which is individually determined. In this connection, it is worth noting that, as remarked previously, gross signs of epithelial secretion in the thymus were detected only in those dystrophic animals severely affected early in life. In particular, the thymuses of two 3-week-old mice which were close to death from the dystrophic process, were totally epithelial in nature and with a marked degree of secretory activity evident.

Table 1 Histological features of thymuses from normal and dystrophic mice

	No. of mice	% normal	Histological features: % without distinct cortico-medullary junction	Histological features: % showing signs of epithelial secretion
+/?	40	90	10	5
dy/dy	45	4	96	29
CBA	10	100	0	0

The percentage of abnormalities seen in clinically normal animals (+/?) may be explained by the uncertainty inherent in selecting as normal all animals which do not show overt clinical symptoms of dystrophy. It is possible that in doing this, animals which are dystrophic but have not yet developed the clinical symptons, may be classified as normal. It is for this reason that the CBA mice were included in this study. Further investigations are being made on the thymus of the homozygous normal Bar Harbor 129/ReJ strain of mice.

The effect of the thymus on immune mechanisms is most marked in mice in neonatal and early postnatal life (up to 4 weeks of age); thereafter the influence of the thymus on immunological capacity declines. Also, the signs of epithelial secretion noted in the thymuses of dystrophic mice at various ages after 2 weeks of age, are similar to those found by Clark in normal mice from birth up to an age of 2 weeks[9].

The alteration of thymic weights have been independently confirmed by D. F. Horrobin and R. A. Karmali (see following paper), who found alterations in the responses of thymuses from dystrophic animals to prolactin, and to the suppression of prolactin secretion.

On the basis of these observations, it is postulated that the thymus in murine muscular dystrophy is functionally altered, and that this alteration probably results in the abnormal secretion of one or more thymic hormones. Whether this altered secretion is due to a malfunction within the thymus itself or to a disorder of a system interacting with the thymus, is not known. This and many other possible avenues are yet to be investigated. Further investigation of the thymic abnormality of dystrophic mice, including periodic acid–Schiff staining and ultrastructural studies, are currently underway.

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Abnormalities of thymus growth in dystrophic mice

In spite of the attention paid to the relationship between myasthenia gravis and the thymus, little work has been done on the thymus in muscular dystrophy. Walker[1] found that mouse dystrophy was not alleviated by neonatal thymectomy and Matheson[2] provided evidence against the idea that muscular dystrophy might be a lymphocyte-mediated autoimmune disease. It therefore seems unlikely that overactivity of lymphoid function of the thymus has any role in muscular dystrophy: the possible role of a thymic deficiency has not been considered. Our interest was aroused when we noticed that in certain superficial ways dystrophic mice of the Bar Harbor 129 strain[3–5] appear similar to mice which have been neonatally thymectomised. Their growth is stunted to a very variable degree, their coat quality is poor and they are liable to die suddenly for no very obvious reason. We now present evidence for an abnormality of thymus growth in these animals.

Initially we simply compared body and thymus weights in untreated 4-week and 12-week-old mice of the Swiss and Bar Harbor 129/ReJ strains. At 4 weeks, 1 week after weaning, it is usually possible to tell by observation whether a 129 animal is dystrophic (dy/dy) or phenotypically normal (+/+ or +/dy). The presence or absence of dystrophy was verified by muscle histology. The results are shown in Table 1. There were approximately equal numbers of each sex in each group and no sex differences were noted. At both 4 and 12 weeks the Swiss mice were significantly larger than phenotypically normal 129 mice ($P<0.01$)—which in turn were larger than the dystrophic mice. At 4 weeks there were no differences in absolute thymus weights between the Swiss and normal 129 mice but in the dystrophic mice the glands were very much smaller ($P<0.001$). At 12 weeks the normal reduction in thymus weight was well advanced in the Swiss mice but less so in the normal 129s. In the dystrophic animals thymus weight was actually greater than at 4 weeks ($P<0.01$). Because of the known effects of adrenal steroids on the thymus, in some of the dystrophic and phenotypically normal 4-week-old animals, we measured adrenal weights as well. In the normals mean weight of the adrenals was 3.4 ± 0.5 (s.e.m.) mg and in the dystrophics it was 1.6 ± 0.3 mg. Adrenal/body weight ratio

Table 1 Body and thymus weights and thymus/body weight ratios in Swiss mice and in dystrophic and phenotypically normal animals of the Bar Harbor 129 strain.

At 4 weeks of age	Swiss	Normal 129	Dystrophic 129
Number	16	8	8
Body weight (g)	21.4±1.36	17.3±0.76	9.3±1.9
Thymus weight (mg)	73.0±5.5	72.2±4.3	3.7±1.2
Thymus/body weight ratio ($\times 10^{-3}$)	3.5±0.3	4.2±0.1	0.37±0.19
At 12 weeks of age	**Swiss**	**Normal 129**	**Dystrophic 129**
Number	16	8	8
Body weight (g)	32.4±0.9	26.2±0.82	16.5±1.1
Thymus weight (mg)	18.0±1.3	32.6±2.0	19.4±3.3
Thymus/body weight ratio ($\times 10^{-3}$)	0.56±0.03	1.25±0.08	1.20±0.28

Results are shown as means ± s.e.m.

in the normals was 3.0×10^{-4} and in the dystrophics 3.1×10^{-4}. Adrenal weight is therefore a normal proportion of body weight in the dystrophic animals and it is unlikely that the thymus atrophy is caused by adrenal hyperactivity.

We then investigated the possibility that thymus growth might be affected at an earlier stage in the dystrophic animals. Three litters were killed between 15 and 20 d *post partum* before weaning. Dystrophic animals were identified by muscle histology. Thymus/body weight ratio in the normal 129s was 3.9×10^{-3} and in the dystrophics 2.3×10^{-3}. Thus the thymus was lower in weight at this stage but the severe reduction noted in the dystrophics one week after weaning was not found.

This pattern is similar to the situation in growth hormone and prolactin-deficient dwarf mice[6-8]. In these animals thymus growth is normal until weaning, apparently being maintained by a factor in the milk. On weaning rapid thymus atrophy occurs. This can be reduced or prevented by treatment with exogenous growth hormone or prolactin. Prolactin is known to be present in rodent milk[9] and it is possible that this is the necessary factor. Quite independently, de Kretser and Livett[10] in Melbourne have made similar observations on thymus growth in dystrophic mice. The interpretation of our common findings must obviously be uncertain at this sage although our own hypothesis is that both muscle and thymus are damaged by the basic genetic defect but that the clinical expression of the genetic defect in the muscles may depend on some of the consequences of thymus damage.

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Foetoneonatal oestradiol-binding protein in mouse brain cytosol is α foetoprotein

Brain cytosol from foetal and neonatal rodents contains high concentrations of an oestradiol-binding protein (foetoneonatal oestradiol-binding protein or FEBP) with properties distinctly different from those of adult oestrogen receptors with respect to sedimentation rate, binding specificity and affinity for oestradiol[1-3]; FEBP declines to undetectable levels by about 22 d in rat brain cytosol[1,2]. Sexual differentiation of the rodent brain, which normally occurs under the influence of testicular secretions between birth and about 10 d, can also be brought about by injection of oestradiol benzoate into neonatal female or castrated male rodents[4]. This observation has led to the idea that FEBP protects the developing brain against the effects of maternal oestrogens[5]. It has been suggested that FEBP in rats is α foetoprotein (AFP)[6], a circulating foetal protein known to bind oestradiol[7-9]. Here we present evidence which points strongly to the identity of mouse FEBP and AFP. This includes a comparison of the binding properties of FEBP with those of purified mouse AFP, correlation of the postnatal disappearance of FEBP with measurements of AFP in brain cytosol and removal of oestradiol-binding activity from brain cytosol using anti-mouse AFP antibodies.

Cytosol—prepared from a homogenate of pooled brains from neonatal mice by centrifugation at 216,000*g*—or mouse AFP—purified by immunoadsorbents[10,11]—was incubated for 2 h at 0 °C with 5×10^{-9} M ^{3}H-oestradiol and centrifuged on glycerol gradients (Fig. 1). The sedimentation coefficients of the peaks of ^{3}H-radioactivity were estimated to be 4.6±0.03S for FEBP (mean ±s.e. of nine determinations involving five preparations from 1–5-d-old mice) and 4.7±0.02S for AFP (mean ±s.e. of 13 determinations on four different AFP preparations). This slight difference in the sedimentation rates of AFP and FEBP could be due to a change that occurred in the AFP molecule as a result of the purification; this was supported by the observation that an AFP preparation purified from brain cytosol gave an S value (4.7 S) comparable with that of the other purified AFP preparations. Figure 1 also shows that excess unlabelled oestradiol (10^{-6} M) decreased binding of ^{3}H-oestradiol to both FEBP and AFP by 80–90%. Diethylstilbestrol (DES), a potent synthetic oestrogen, at 10^{-6} M, had no effect on oestradiol binding to FEBP, in agreement with results reported for rat FEBP[1,2]; 103% (mean of four determinations) of the ^{3}H-c.p.m. found in this peak in the absence of DES was bound in its presence. DES competed slightly, however, with ^{3}H-oestradiol for binding to purified mouse AFP (17%, mean of four determinations); ^{3}H-DES has also been reported to bind to purified rat AFP[9]. The apparent difference in sensitivity to DES competition between FEBP and AFP was abolished when purified AFP

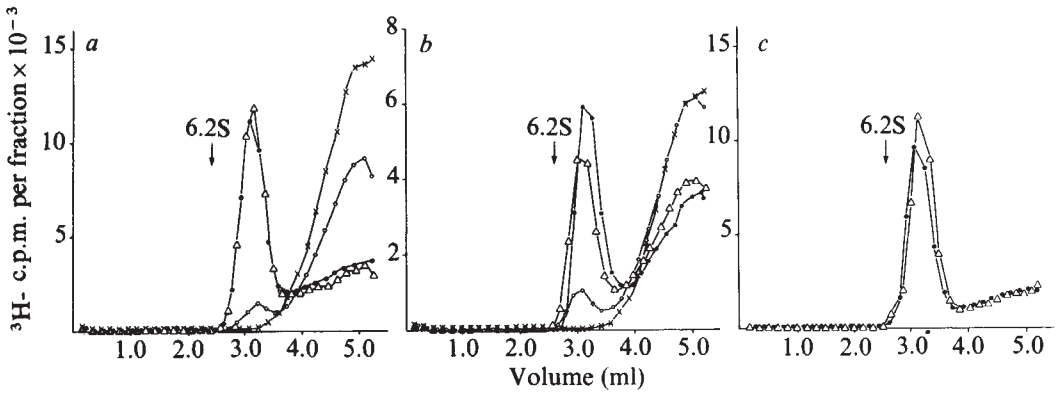


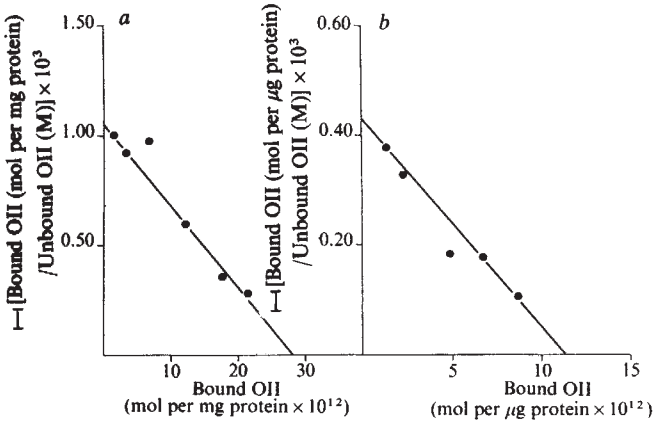
Fig. 1 Glycerol gradient centrifugation of 5-d brain cytosol and mouse AFP incubated with ^{3}H-oestradiol and ^{3}H-testosterone. Cytosol was prepared from pooled brains of 5-d-old female mice or from 3–5-month-old adult mice in TEM (0.01 M Tris, *p*H 6.7, at 25 °C; 0.0015 M EDTA; 0.002 M β-mercaptoethanol) by centrifugation for 1 h at 216,000*g* as described before[13]. Mouse AFP, purified by immunoadsorbents[10,11], was dissolved in TEM. Samples of 0.4 ml of 5-d cytosol (4.7 mg per ml of protein) (*a*), of AFP (18 μg ml^{-1}) (*b*), or of AFP (18 μg ml^{-1}) in adult cytosol (6.8 mg per ml of protein) (*c*) were incubated with 5×10^{-9} M ^{3}H-17β-6,7-oestradiol (47.9 Ci mmol^{-1}) in the absence (●) or presence of unlabelled oestradiol at 10^{-6} M (○) or DES at 10^{-6} M (△), and with 5×10^{-9} M ^{3}H-1α,2α-testosterone (59 Ci mmol^{-1}) (×) for 2 h at 0 °C. Samples were layered on 5–35% linear glycerol gradients in TEM and centrifuged in the SW 50.1 rotor at 216,000*g* for 16 h at 2 °C. The scale on the left of (*b*) refers to the gradients containing ^{3}H-oestradiol, while that on the right refers to the gradient containing ^{3}H-testosterone. The arrow indicates the position of bacterial alkaline phosphatase added as an internal sedimentation marker (6.2S, see ref. 13). The direction of sedimentation was from right to left.

was incubated with ^{3}H-oestradiol in the presence of brain cytosol from adult mice (which contains no detectable FEBP). DES no longer displaced any radioactivity from the AFP peak (Fig. 1); in two such experiments, 104% and 109% of the ^{3}H-c.p.m. were recovered in the AFP peak in the presence of DES. There was no indication of binding of ^{3}H-testosterone to either FEBP or mouse AFP (Fig. 1).

Binding parameters for the reaction of brain cytosol or mouse AFP with ^{3}H-oestradiol were obtained by Scatchard[12] analysis after equilibrium dialysis. This method was chosen since we[13] and others[14] have observed that there is a rapid dissociation of the FEBP-oestradiol complex in non-equilibrium conditions. The mean dissociation constants (k_D) from two experiments were 2.3×10^{-8} M (brain FEBP) and 2.4×10^{-8} M (mouse AFP); these are essentially the same as published values for rat brain FEBP[1] and purified rat AFP[9].

AFP was demonstrable in brain cytosol from immature mice by immunodiffusion or radioimmunoassay[10]. Cytosol from 1-, 4–5-, 9–10- and 20–22-d-old mice gave AFP concentrations (mean ±s.e.) of 7.0±0.8 μg (seven determinations), 1.5±0.2 μg (three determinations), 0.24±0.02 μg (three determinations), and <2 ng (undetectable level) per mg of protein, respectively. There were 0.7–0.8 mol of oestradiol bound per mol of mouse AFP (molecular weight 74,000 (ref. 10)), and the binding capacity of brain cytosol from 4-d-old mice was about 25 pmol per mg of protein (Fig. 2). Assuming the binding moiety in brain cytosol to be AFP with one binding site per molecule, 4-d brain cytosol should contain 1.8–1.9 μg of

Fig. 2 Scatchard plots representing binding of ^{3}H-oestradiol by 4-d brain cytosol (*a*) and mouse AFP (*b*). Samples of 0.35 ml of brain cytosol from 4-d-old male mice (11 mg per ml of protein) or of AFP (17 μg ml^{-1}) were dialysed against 10 ml TEM containing various concentrations of ^{3}H-oestradiol (OII) (2×10^{-9}–1×10^{-7} M) for 24 h at 4 °C. Triplicate samples of the inner and outer solutions were removed for counting. Bound radioactivity was determined from the difference between the radioactivity inside the dialysis bags and that outside.



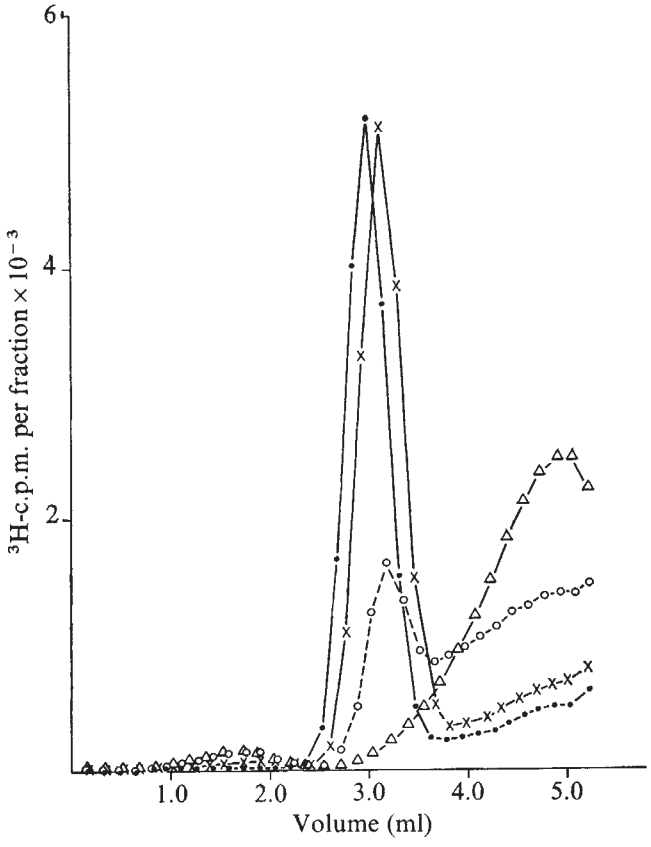


Fig. 3 Glycerol gradient analysis of brain cytosol from mice of various ages incubated with ^{3}H-oestradiol. Cytosol from two or three female mice aged 1 (●), 4 (×), 10 (○) and 21 d(△) was incubated with 2×10^{-9} M ^{3}H-oestradiol. Samples of 0.4 ml containing 1.6, 2.6, 3.2, and 3.7 mg of protein, respectively, were centrifuged on glycerol gradients as in Fig. 1.

AFP per mg of protein. This is in close agreement with the observed AFP concentration (1.5 μg per mg of protein) at this age. The time course of disappearance of 4.6S oestradiol-binding activity in brain cytosol (Fig. 3) parallels the decrease in AFP levels. At 1 and 5 d substantially all the ^{3}H-radioactivity was found in the 4.6S peak (at 5×10^{-9} ^{3}H-oestradiol). At 10 d there was an appreciable decrease in the proportion of bound ^{3}H-oestradiol, with a concomitant increase in free steroid at the top of the gradient, and by 21 d no binding was detectable.

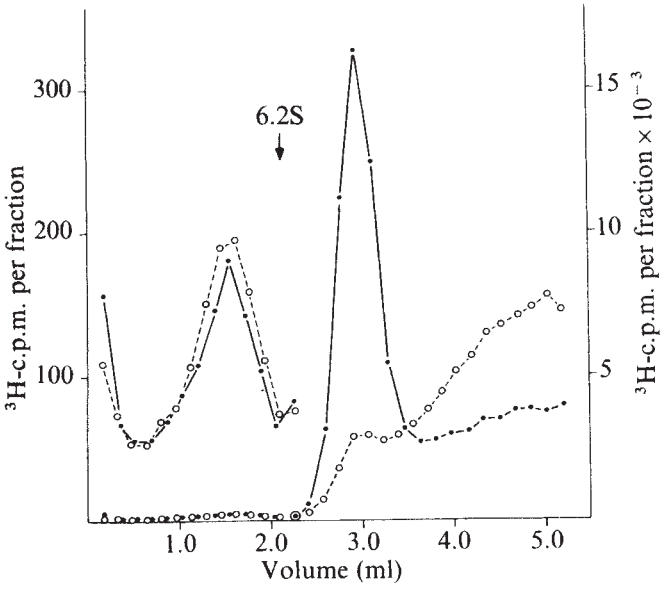


Fig. 4 Glycerol gradient analysis of brain cytosol from 5-d-old male mice incubated with ^{3}H-oestradiol after treatment with anti-mouse AFP or an unrelated control adsorbent. Cytosol was treated with Sepharose-coupled anti-mouse AFP antibodies (○) or with control adsorbent (●) for 3 h at 0 °C and incubated with 5×10^{-9} M ^{3}H-oestradiol. Samples of 0.5 ml containing 2.5 and 2.2 mg of protein, respectively, were centrifuged on glycerol gradients as in Fig. 1. The scale on the left refers to the expanded plot of the bottom part of the gradients showing the peaks of 8S oestradiol receptor.

Treatment of 5-d brain cytosol with anti-mouse AFP antibodies insolubilised on to Sepharose removed almost completely the 4.6S oestradiol-binding activity, while this binding was not affected in a sample treated with an unrelated control Sepharose adsorbent (Fig. 4). The binding data were in agreement with measurements of AFP in these samples: these were 0.12 and 1.4 μg of AFP per mg of protein, respectively. In contrast, there was no change in the oestradiol-binding capacity of the 8S oestradiol receptor[13] after exposure of cytosol to anti-mouse AFP. A second experiment involving 1-d brain cytosol gave essentially the same results.

Thus FEBP in neonatal mouse brain cytosol has properties similar to those of purified mouse AFP with respect to affinity for 17β-oestradiol, specificity of the binding reaction and sedimentation rate in glycerol gradients. In addition, AFP can be detected in cytosol preparations by immunodiffusion or radioimmunoassay, and AFP and 4.6S oestradiol-binding activity show a similar time course of disappearance in the developing brain and coincident removal from brain cytosol by anti-mouse AFP antibodies. By all these criteria AFP and FEBP are substantially identical.

The biological role of AFP is unclear, but its high capacity for oestradiol (and oestrone) has led to the idea that it may be involved in the protection of foetal tissues against circulating maternal oestrogens[1,5]. This may be particularly important in the brain because the FEBP-AFP concentration in brain cytosol seems to be higher than can be accounted for by contamination from the blood[1,6]. The oestrogen-binding properties of AFP, however, may be confined to rodents and this may not be the only function of this protein. This is suggested by the lack of significant oestrogen binding, demonstrable by equilibrium dialysis or gradient centrifugation, by human AFP-containing serum or amniotic fluid[15,16] or by purified human and bovine AFPs (our unpublished results). Also AFP shows sequence homology with serum albumin[17], and oestrogen binding may be only a specialised expression of general albumin-like binding properties of the AFP molecule.

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Inhibition of platelet aggregation by a myeloma protein with anti-phosphocholine specificity

PHOSPHOCHOLINE-containing phospholipids, particularly phosphatidylcholine (lecithin), lysophosphatidylcholine and sphingomyelin, have integral roles in the structure and function of mammalian cell membranes[1]. Enzymatic digestion of platelet choline phosphatides, particularly of phosphatidylcholine which is the most abundant of the platelet phospholipids[2,3], has been shown to induce platelet release reactions[4]. We have demonstrated that C-reactive protein (CRP), an acute phase reactant with binding specificities for phosphocholine, choline phosphatides and polycations generally[5–8], can inhibit multiple platelet reactivities[9,10]. These considerations have raised the possibility that molecules generally with binding specificities for choline phosphatides can influence platelet responsiveness. We report here an investigation of the ability of the T-15 anti-phosphocholine mouse IgA myeloma protein to inhibit the aggregating response of human platelets.

Diluents and human platelets were prepared and platelet viability and aggregation were assayed as described before[9]. The IgA-producing plasma-cell tumours TEPC-15 and MOPC-315 were provided by Dr Donald A. Rowley, University of Chicago, and were maintained in BALB/cJ mice (Jackson Laboratories). The anti-phosphocholine myeloma protein T-15 was isolated by affinity chromatography from pools of ascites fluid obtained from tumour-bearing mice essentially by the method of Chesebro and Metzger[11], except

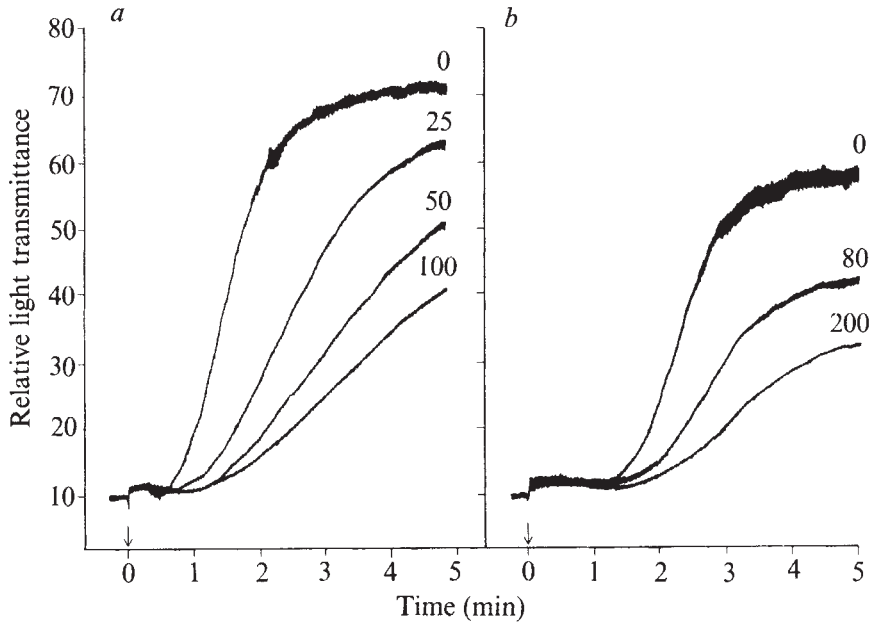

Fig. 1 The effects of increasing amounts of T-15 on platelet aggregation induced by thrombin (0.2 units ml^{-1}) (*a*) and AHGG (50 $\mu g\ ml^{-1}$) (*b*); the concentrations of T-15 added to the reaction mixtures are expressed in $\mu g\ ml^{-1}$.

that C-polysaccharide–Agarose beads[12] were used in place of phosphocholine–Agarose. The protein was purified further by filtration on BioGel A-0.5 m, and the material reactive with rabbit anti-mouse IgA antiserum corresponding to the 11S dimer was used. Similarly, the anti-dinitrophenol myeloma protein M-315 was isolated from pools of serum and ascites fluid obtained from mice bearing MOPC-315 by affinity chromatography on trinitrophenylaminoethyl Agarose beads (BioGel, A-15 m); it was eluted with 0.1 M dinitrophenol, and the dinitrophenol was removed on Dowex 1 (ref. 13).

The T-15 mouse IgA myeloma protein reactive with phosphocholine was found to inhibit the ability of washed human platelets to aggregate in response to thrombin and aggregated human γ globulin. This inhibition was characterised by a reduced rate (slope) and extent (amplitude) of aggregation, and a decreased size of the platelet aggregates formed (magnitude of the vertical oscillations); concentrations as low as 25–80 $\mu g\ ml^{-1}$ were sufficient to bring about these effects (Fig. 1).

By contrast, the M-315 mouse IgA myeloma protein did not inhibit aggregation at concentrations as high as 425 $\mu g\ ml^{-1}$, indicating that the inhibition by T-15 involved its antigen-combining site. In support of this interpretation, inhibition by T-15 was reduced substantially when phosphocholine was included in the reaction mixtures (Table 1).

Table 1 Effect of phosphocholine on T-15–inhibited aggregation of platelets stimulated by thrombin and AHGG

Inhibitor	% Inhibition ± s.d.* Thrombin	AHGG
T-15	56±10.0	54±7.1
T-15+PC	15±7.1	26±1.4
PC	1±0.5	2±1.4

Platelets (3×10^9) were equilibrated with T-15 for 1 min at 37 °C and challenged with thrombin (0.2 units ml^{-1}) or AHGG (50 $\mu g\ ml^{-1}$); aggregation was recorded for 5 min. The concentration of T-15 chosen was sufficient to inhibit the rate of aggregation by approximately 50% and represents 60 (thrombin) and 80 (AHGG) $\mu g\ ml^{-1}$; phosphocholine (PC) was at a final concentration of 10^{-3} M.

*The data are presented as the percentage inhibition in the rate of platelet aggregation ± 1 standard deviation (s.d.) as compared to the aggregation response obtained in the absence of inhibitors.

The possibility that T-15 acts by reducing platelet viability was discounted because concentrations far in excess of those used in this study (>950 $\mu g\ ml^{-1}$) were not cytotoxic as determined by incorporation of ^{14}C-5-hydroxytryptamine (^{14}C-5-HT) and release of lactic dehydrogenase.

Thus, the T-15 myeloma protein with anti-phosphocholine specificity inhibits the reactivity of human platelets with characteristics similar in all respects investigated to those previously observed when CRP was added to these mixtures. It had not been clear whether CRP reacts with phosphocholine lipids, polycations or other of its binding specificities to cause its inhibitory effect. Our data suggest that a phosphocholine moiety on the platelet surface is involved in this inhibition, and that phosphocholine-binding molecules generally can modulate platelet reactivities; CRP would seem to be biologically the most important of the latter. The anti-phosphocholine myeloma protein may well aid in the delineation of the surface receptors and pathways involved in the CRP-mediated control not only of platelet reactivities, but also of responses of other cells reactive with CRP, such as subpopulations of T cells[14–16]. The nature of these receptors, and the significance of the association of molecules with which they interact with inflammation and tissue destruction, seem to be critical to a more complete understanding of body defence mechanisms.

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Antiviral antibodies inhibit the lysis of tumour cells by anti-H–2 sera

RECENT experiments have indicated that the cytolysis of virus-infected, chemically modified, or neoplastic cells by murine T lymphocytes is restricted to target cells carrying one or more of the H–2 antigens possessed by the cells against which the cytotoxic T cells were generated[1-10]. Two general hypotheses have been proposed to explain the participation of H–2 antigens in T–cell-mediated cytolysis. According to one hypothesis, T lymphocytes possess two different types of receptors, one which recognises the abnormal antigens of the target cell, and a second which interacts with the H–2 antigens of that cell. In the alternative hypothesis, the T cell has only one specific receptor that is directed against a complex antigenic determinant made of of H–2 antigens and viral antigens, for example, tumour antigens induced by viral infection. Neither of these hypotheses has yet been satisfactorily proven. In support of the one-receptor hypothesis, we have found that anti-H–2 and antiviral sera show copatching and cocapping of H–2 and viral antigens on the surface of EL4 leukaemia cells[9], suggesting that viral and H–2 antigenic determinants can be in close physical association on the cell surface. Here we provide new evidence for the existence of a close association between H–2 antigens and viral antigens on murine leukaemia cells.

We have used the method of antibody-induced resistance to complement-dependent lysis ("lysostrip" technique[11]) to show that the binding of antiviral antibody to the cell surface resulted in resistance to cell lysis induced by subsequent treatment with anti-H–2 serum and complement. Bernoco *et al.*[11] and Hauptfeld *et al.*[12] have shown that specific resistance to lysis by an alloantiserum can be effected by first coating cells with the alloantiserum and then incubating the cells with an anti-Ig serum directed against the first antibody.

Treatment of EL4 cells (H–2^b) with a rabbit antiserum to Rauscher leukaemia virus (RLV), or with a goat antiserum against the purified glycoprotein gp70 of murine leukaemia virus (Scripps), and then with an appropriate anti-Ig reagent reduced the subsequent complement-mediated lysis of the cell by anti-H–2^b antiserum (Table 1). In contrast, both of the antiviral antisera had only slight and inconsistent effects in similar experiments using normal lymphocytes from the parental mouse strain C57BL/6 (Table 1). The corresponding control sera (normal rabbit serum taken before immunisation with RLV or pooled normal goat serum) had no effect either on EL4 cells or spleen cells. Before use, all of the heterologous sera were absorbed at least twice with equal volumes of normal spleen cells.

The efficiency and specificity of the "lysostrip" technique was demonstrated on both normal spleen cells and tumour cells. Pretreatment with antisera to the H–$2D^b$ antigens caused no reduction or very little reduction in the susceptibility to lysis by an anti-H–$2K^b$ antisera (Table 1). Similarly, precoating the cells with antiserum to the H–$2K^b$ antigens caused no reduction in susceptibility to lysis by anti-H–$2D^b$ serum (Table 1). Precoating the cells with an anti-H–$2K^b$ antiserum, however, did lead to an increased lysis in the cytotoxicity assay performed with an anti-H–$2D^b$ antiserum. This increase was probably due to anti-H–$2K^b$ antibodies that had bound to the cells and were present during the cytotoxic assay. It was not observed if the precoated cells were treated with goat antibody to mouse IgG, which also suggests that it was due to anti-H–$2K^b$ antibodies that were bound in the first step of the experiment.

These results confirm that resistance to lysis can be induced only by redistribution of the H–2 antigen specifically tested in the cytotoxicity assay, as reported by Hauptfeld *et al.*[12]. The observation that the pretreatment of EL4 cells with antiviral sera induced a partial resistance to cytotoxicity mediated by alloantisera against both the K and the D end of the major H–2 complex is therefore not likely to be a peculiarity of the method that we used.

The identity of the relevant viral antigens carried by the EL4 cells is unknown. Sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis of immune precipitates made from ^{125}I-labelled EL4 cells using RLV, however, showed a major component corresponding in mobility to the viral glycoprotein gp70 (Fig. 1). Similar results were obtained with goat antisera against the gp70 glycoproteins of Rauscher leukaemia virus[13] or murine leukaemia virus (Scripps). All three antiviral antisera also stained the EL4 cells brightly by immunofluorescence. Viral antigens were not detected on C57BL/6 spleen cells with these antisera by either immunofluorescence or immune precipitation.

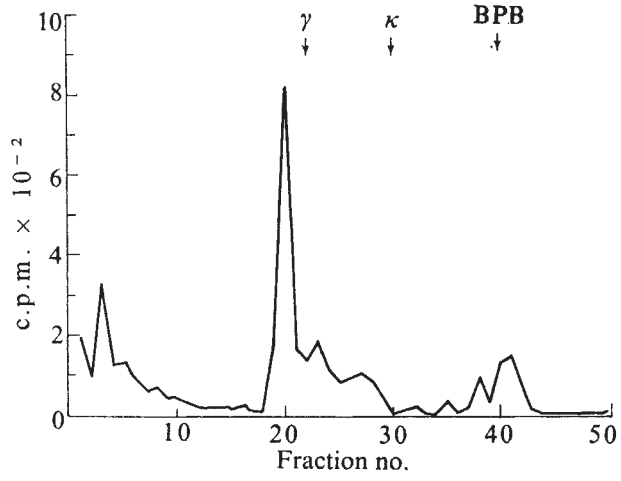

Fig. 1 SDS–polyacrylamide gel electrophoresis of an immunoprecipitate of an extract of ^{125}I-surface-labelled EL4 cells. EL4 cells were iodinated with ^{125}I by the lactoperoxidase technique[21] and extracted with 0.5% NP40 (ref. 19). Immunoprecipitates were obtained using BALB/c absorbed RLV and unabsorbed GARIgG. SDS gel electrophoresis was performed in 8.5% polyacrylamide gels after solubilisation and reduction of the precipitates by boiling for 5 min in sample buffer containing 4% SDS and 5% mercaptoethanol[19]. MOPC 21 myeloma immunoglobulin labelled with ^{131}I was used as marker. γ, Heavy chain (molecular weight, 50,000); κ, light chain (molecular weight, 23,000); BPB, bromophenol blue marker.

Of the artefacts that might arise in these experiments, the most obvious would be ascribable to antiviral antibodies in the anti-H–2 antisera[13-15] or anti-H–2 antibodies in the antiviral antisera. Several kinds of control experiments were performed to explore these possibilities. Most

Table 1 Effect of antiviral sera on cytolysis by anti-H-2 sera

Antisera used in:—		% Specific lysis										
		EL4								C57BL/6		
Step I	Step II	K^{b*}	K^b	K^b	K^b	K^b	K^b†	D^b	D^b	K^b	K^b	D^b
None	None	33	25	33	45	35	61	23	22	34	41	23
RARLV	None	35			45			30	28	34	41	30
RARLV	GARIgG	21			34			19	15	33	39	35
NRS	None	31						29	29	28		37
NRS	GARIgG	33						30	32	28		37
None	GARIgG	39						28	26	29	40	37
GAgp70	None		22	29	40	36				28		
GAgp70	RAGIgG		12	16	16	26				33		
NGS	None		23	33						24		
NGS	RAGIgG		27	31						29		
None	RAGIgG			33		34				23		
Anti-H-$2D^b$	None						71	33	32	34		20
Anti-H-$2D^b$	GAMIgG						65	16	13	30		3
Anti-H-$2K^b$	None	39	27	34	49	35	61	42	39	37	40	37
Anti-H-$2K^b$	GAMIgG	11	8	12	9	15	39	33	30	8	−1	22
None	GAMIgG	26				43		29	23	31	39	23

RLV, rabbit antiserum against Rauscher leukaemia virus; GARIgG, goat antiserum against- rabbit IgG; NRS, normal rabbit serum; GAgp70, goat antiserum against gp70; RAGIgG, rabbit antiserum against goat IgG; NGS, normal goat serum; GAMIgG, goat antiserum against mouse IgG. The procedure was performed in three steps. Step I. 5×10^5 ^{51}Cr-labelled cells were coated with 0.02 ml antiviral or anti-H-2 antibody in 0.2 ml Minimal Essential Medium with 5% heat-inactivated foetal bovine serum (MEM/FBS) for 30 min on ice. Step II. After washing twice, cells were incubated for 45 min at 37 °C in 0.2 ml MEM/FBS with 0.02 ml of a second antiserum directed against the first antibody. Cells were washed once and this step was repeated. After completion of this second cycle cells were washed once and resuspended in 0.22 ml MEM/FBS. Step III. Cytotoxicity assay was performed using 2×0.05 ml of this cell suspension as described earlier[19] using antisera directed against the H-$2K^b$ or H-$2D^b$ antigens as indicated (*). Total c.p.m. were determined in 2×0.05 ml of the same cell suspension. Results are given in percentage specific lysis calculated according to the following formula:

$$\frac{\text{Experimental c.p.m.} - \text{background c.p.m.}}{\text{Total releasable c.p.m.} - \text{background c.p.m.}} \times 100$$

Background lysis was determined separately and varied between 14 and 30%. Each antiserum used was also tested individually to exclude nonspecific lysis due to incomplete absorption. EL4 cells (Salk Institute) were grown in ascitic form in C57BL/6 mice (Jackson Laboratory). Splenic lymphocytes were prepared by the Ficoll–Hypaque technique[20]. Cells were labelled with 100 μCi of ^{51}Cr per 10^7 cells. Antisera used in step I were anti-H-$2K^b$ (H-2.33) and anti-H-$2D^b$ (H-2.2) prepared as described earlier[19] and were used undiluted. RLV was produced by repeated injections of SDS-solubilised Rauscher leukaemia virus (Litton Bionetics) adding complete Freund's and incomplete Freund's adjuvant, respectively, in the first two injections. RLV was absorbed twice with equal volumes of packed C57BL/6 spleen cells or with BALB/c spleen cells (H-2^d) when used on C57BL/6 spleen lymphocytes. NRS was obtained from the rabbit producing the RLV before the first immunisation and was absorbed by the same procedure. GAgp70 raised against gp70 isolated from murine leukaemia virus (Scripps), and pooled NGS were absorbed in the same way as the rabbit sera. Antisera used in step II were GAMIgG (Miles–Yeda, Rehovot) was absorbed with BALB/c thymus cells. GARIgG prepared by repeated injections of rabbit IgG was absorbed with BALB/c spleen and thymus cells, and RAGIgG (Cappel Laboratories) was absorbed with BALB/c spleen cells. In the cytotoxic assay the antiserum against H-$2K^b$ was used at a dilution of 1:18 (except K^b†, dilution: 1:12) and the antiserum against H-$2D^b$ was used at 1:8. Guinea pig complement (Grand Island Biologic. Co., Grand Island) was absorbed with mouse spleen cells and diluted 1:4. Each vertical column represents a single experiment. Results indicating that pretreatment has reduced the susceptibility to complement-mediated lysis by anti-H-2 sera are underlined. When anti-H-$2D^b$ serum was used in the cytotoxicity assay, there was increased specific lysis of cells that had been pretreated with RLV or anti-H-$2D^b$ serum alone. For this reason, the lysostrip effect is probably best estimated by comparing the specific lysis of cells pretreated with the first antibody and then anti-Ig antibodies, with the specific lysis of cells pretreated only with the first antibody or only with anti-Ig antibodies.

mouse alloantisera contain antibodies directed against murine leukaemia antigens[14,15] and particularly against the envelope protein gp70 (refs 13-15). The cytotoxic activity of certain alloantisera against tumour cells may therefore consist of two components, one due to antibodies against alloantigens and the other to antibodies against viral antigens. Accordingly, the anti-H-2^b sera used in this study were absorbed three times with equal volumes of packed thymocytes of young BALB/c mice (H-2^d) or with NZB-thymoma cells that were actively producing murine leukaemia virus (Scripps) in large amounts (SCRF 60A, H-2^d). After absorption, the titres of the anti-H-$2K^b$ and anti-H-$2D^b$ sera were compared using EL4 cells as targets. There was no difference in the cytotoxic titres after absorption with either normal thymocytes or virus-producing tumour cells.

This result indicated that our cytotoxicity assay did not detect anti-viral antibodies in the anti-H-2 antisera. It is therefore unlikely that the decreased cytotoxic effect of the anti-H-2 antisera after redistribution of viral antigens merely reflects the absence of cytolysis that might have been due to the presence of antiviral antibodies in the anti-H-2 antisera. It should be noted that small amounts of anti-viral antibodies were present in the anti-H-$2D^b$ antiserum, however, inasmuch as a gp70 component was observed in the SDS-gel electrophoretic analysis of immune precipitates made from ^{125}I-labelled EL4 cells or C57BL/6 spleen cells[13]. On the other hand, immune precipitates using the anti-H-$2K^b$ antiserum on extracts of EL4 or C57BL/6 cells did not contain detectable amounts of viral antigens[13].

The specificity of one of the anti-viral antisera used (RLV) was demonstrated by comparing the "lysostrip" effect of RLV on EL4 cells after absorption with either C57BL/6 spleen cells or with the cell line SCRF 60A (H-2^d) that produces murine leukaemia virus (Scripps). Pretreatment with 4 or 0.8 μl of the RLV that had been absorbed with SCRF 60A (H-2^d) cells did not reduce subsequent cytolysis by H-$2K^b$ antiserum in contrast to pretreatment with similar amounts of the RLV that had been absorbed with C57BL/6 spleen cells (Table 2). The difference between the absorption with virus-producing cells and with normal spleen cells suggests that the effect of RLV in the lysostrip assay is due to antibodies directed against viral antigens rather than to antibodies against H-2 antigens. Similar experiments showed that absorption of the H-$2K^b$ antiserum with C57BL/6 spleen cells completely abolished its ability to induce resistance against anti-H-$2K^b$ mediated cytotoxicity.

These observations confirm and extend our previous

observations on the cocapping of H–2 and viral antigens[9] using the same cell type (EL4) and similar antisera. Both sets of data are explicable in terms of a close physical association or inter-action of viral and H–2 determinants on the cell surface. Because the exact mechanism of the lysostrip phenomenon is not understood, however, the present results are not conclusive evidence for the close molecular association of viral and H–2 determinants in all conditions. Obviously, more direct approaches are required to demonstrate an association at the molecular level.

Although the evidence that viral and H–2 determinants may be associated on the cell surface is only indirect, it is difficult to account for by any other means. It is therefore pertinent to consider the possible mechanism of this molecular association. One possibility is that both viral and H–2 determinants are on one molecule[16]. There are no biochemical data to favour this model, but it cannot be completely excluded at present. We have observed, however, that target cells coated with inactivated Sendai virus are killed by specific cytotoxic T cells within the first 30 min after the cells are coated with the virus[2]. These experiments suggest that any synthetic modification of the H–2 antigens that might occur must be induced very rapidly, a finding that further weakens the hypothesis.

A second possibility, which we favour[9], is that viral and H–2 antigens are in close physical association on the cell membrane. The molecular interaction between the H–2 and viral antigens could be related to a specialised function of either of the molecules. For example, H–2 antigens could have a broad binding specificity for a wide range of viral antigens or could serve as receptors for viruses. Alternatively, H–2 antigens may have a tendency to bind weakly to many surface proteins. This might account for the finding that T-cell-mediated cytotoxicity against minor histo-compatibility antigens also seems to involve the H–2 antigens[17,18]. Although the direct demonstration of hybrid antigens does not in itself exclude the hypothesis that the T cell has two separate recognition sites, one for H–2 and the other for viral antigens, a variety of experiments favours the idea that the T cell has a single kind of receptor for recognition of hybrid antigens.

Table 2 Effect of absorption of RLV with a cell line producing murine leukaemia virus (Scripps)

RLV (μl)	% Specific lysis of targets pretreated with RLV absorbed with C57BL/6 spleen	SCRF 60 A
None	35	35
20	18	19
4	25	35
0.8	27	35
0.4	38	36

RLV was absorbed twice with equal volumes of packed SCRF 60A cells (grown in RPMI 1640 medium with 5% FBS) or with C57BL/6 spleen cells (H–2^b). The lysostrip technique was performed on EL4 cells as described in the legend of Table 1 using the indicated volumes of the absorbed RLV sera in the first step, and GARIgG in the second step. Sensitivity to cytotoxicity was assayed using anti-H–2K^b (1:18). Specific lysis was calculated as described in Table 1. The specific lysis of control cells pretreated only with RLV and not with GARIgG was 43% for the RLV absorbed with C57BL/6 cells, and 40% for the RLV absorbed with SCRF 60A cells.

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GABA-mediated control of rat neostriatal tyrosine hydroxylase revealed by intranigral muscimol

BLOCKADE of central dopamine (DA) receptors by neuroleptic drugs is associated with an increase in the firing rate of nigro-neostriatal DA neurones[1] and an enhanced turnover rate of DA in neostriatum (caudate–putamen)[2,3].

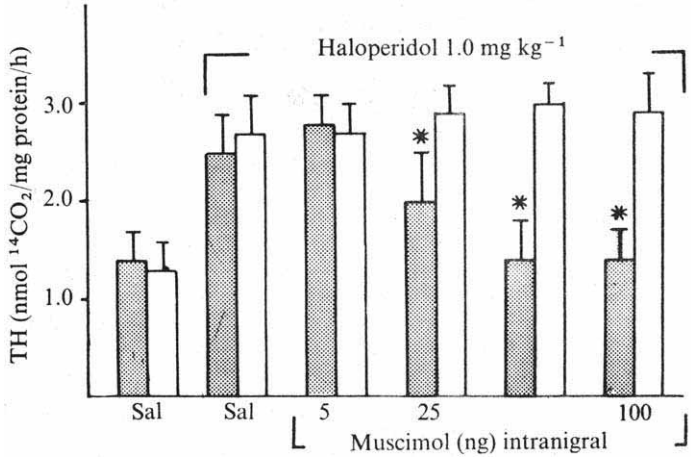

Fig. 1 Tyrosine hydroxylase (TH) activity from the right (open bars) and left (darkened bars) caudate–putamen of rats treated with saline (Sal) or muscimol directly into the left substantia nigra by way of chronically implanted stainless-steel cannulae. Muscimol was dissolved in saline and administered in a volume of 0.3 μl, over a 3-min period. Ten minutes after intranigral injection, haloperidol (1.0 mg kg^{-1} dissolved in 0.05% acetic acid, intraperitoneally) or its vehicle alone (first pair of bars) was administered intraperitoneally. Animals were decapitated 40 min after intranigral injection and the tissue to be assayed was rapidly dissected out and frozen on dry ice. Assay for TH was done according to the method of Zivkovic *et al.*[5]. Values shown were obtained using 0.3 mM DMPH$_4$ cofactor and 0.1 mM tyrosine. Each value represents the mean of eight rats (bar indicates s.e.). *$P < 0.05$ when compared with the contralateral (control) side.

At the molecular level, the neuroleptic-induced increase of caudate–putamen (CP) DA metabolism is maintained by a decrease in the K_m of tyrosine hydroxylase (TH), for pteridine cofactors[4-6]. This increase in affinity of TH for cofactor occurs within 20 min of a single injection of a neuroloptic such as haloperidol or pimozide and lasts for at least 2 h. The action of haloperidol and other neuroleptics on CP–TH activity is abolished by either mechanical (lesion) or functional (γ-butyrolactone) interruption of nigro-neostriatal DA projections[7-9]. It is likely, therefore, that the neuronal "feedback loop" hypothesis, proposed originally by Carlsson and Lindqvist[3] to explain the regulation of nigro-neostriatial DA biosynthesis, is also relevant to the acute activation of CP–TH after systemic administration of neuroleptics. According to this theory, a neural pathway from neostriatum to substantia nigra (SN) is responsible for mediating an increase in presynaptic DA function in an effort to compensate for reduced neostriatal postsynaptic DA receptor activity.

Histochemical studies have demonstrated the presence of a GABA-containing pathway originating in the caudate nucleus with terminal projections in close proximity to soma and dendrites of nigral DA neurones[10-14]. Electrophysiological evidence indicates that the GABAergic neurones in the striatonigral pathway exert a picrotoxin-sensitive, inhibitory influence on nigral cells[15]. It is possible that a reduced release of GABA from these descending inhibitory neurones may occur as a result of neuroleptic blockade of CP–DA receptors. Activation of CP–TH could then occur as a consequence of the decreased GABAergic inhibitory control of nigral DA neurones.

The experimental verification of this hypothesis was made possible by the use of muscimol. This isoxazol derivative, with a restricted conformation similar to that of GABA, displays bicuculline-sensitive and strychnine-insensitive inhibitory activity both *in vivo* and *in vitro*[16,17], but unlike GABA is not rapidly metabolised and thus exhibits long lasting GABA-mimetic effects *in vivo*[18]. Moreover, on the basis of experiments with a DA-sensitive adenylate cyclase, we have established that this compound is devoid of any DA-agonist or antagonist potency in homogenates of neostriatum or substantia nigra.

Muscimol was injected directly into the SN by way of chronically implanted cannulae (for detail see Figs 1 and 2). Ten minutes later haloperidol (1.0 mg kg^{-1}) was administered intraperitoneally and the animals were killed 30 min later for TH assay. In this experiment, TH was assayed using a concentration of cofactor ($DMPH_4$) below that required to saturate the enzyme fully.

Figure 1 shows that the haloperidol-induced increase of CP–TH activity was blocked in the caudate ipsilateral to the intranigral application of muscimol. A 50% reduction of the action of haloperidol was obtained by injection of 25 ng of muscimol into the SN; 50 ng of muscimol reliably and completely prevented the action of haloperidol on CP–TH. Muscimol (50–400 ng) alone did not alter normal CP–TH activity when measured at 20 and 40 min after intranigral injection.

The differences in the values for TH activity presented in Fig. 1 were found to reflect differences in the apparent K_m values of TH for the pteridine cofactor. Double-reciprocal plots of TH activity obtained in the presence of different cofactor concentrations and K_m values for normal, haloperidol-"activated", and muscimol-"protected" CP–TH

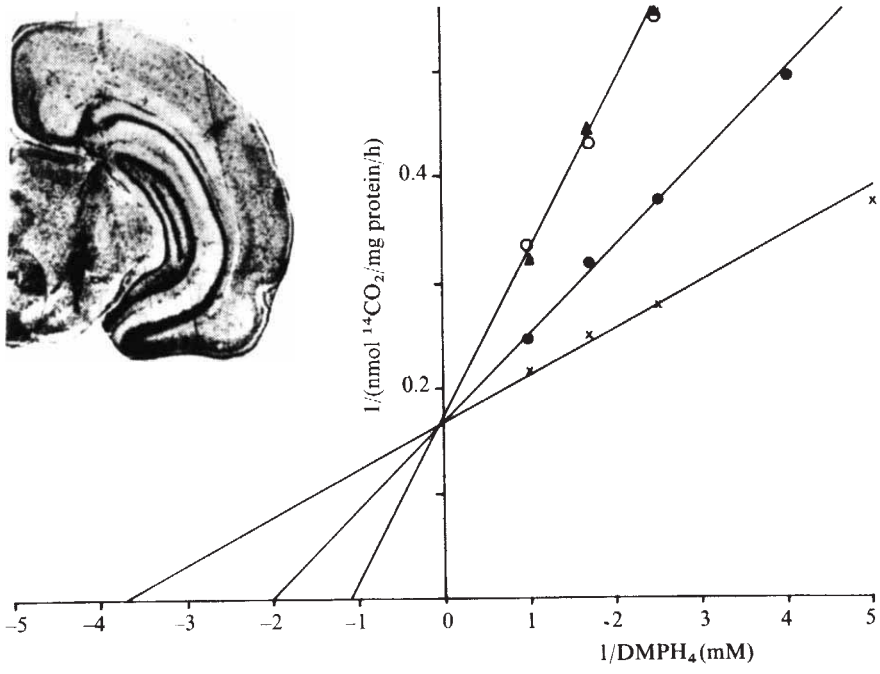

Fig. 2 Double-reciprocal plots of the initial velocity of CP–TH against various concentrations of $DMPH_4$ in the presence of 0.1 mM tyrosine. Experimental design was as described in Fig. 1. Muscimol (○, 50 ng; ●, 25 ng) or saline (×) was injected into the substantia nigra (SN). Ten minutes after intranigral injection, haloperidol (1 mg kg^{-1}) was administered intraperitoneally. The animals were killed 40 min after the intranigral injection. Control rats (▲) received saline intranigrally and vehicle intraperitoneally. Insert: photomicrograph of coronal section of rat brain (left hemisphere) showing position of (22 g) guide-cannula tip (blackened area above the dorsal border of the SN). Injection cannula (27 g) extended 0.8 mm below guide cannula into the pars compacta region of the SN.

are shown in Fig 2. It is clear that intranigral muscimol treatment (50 ng or more) prevented the marked decrease (two- to three-fold) in apparent K_m caused by haloperidol treatment. Similar results were obtained with pimozide (1.0 mg kg^{-1}) treatment.

To determine whether the effect of intranigral muscimol was specific for the DA neurones projecting to the neostriatum, we assayed the TH activity of the nucleus accumbens of eight rats which received 25 ng of intranigral muscimol and 1.0 mg kg^{-1} haloperidol intraperitoneally. In these animals, the percentage differences between the two neostriata averaged 55% (range 40–70%), with the muscimol-treated side exhibiting lower TH activity. In contrast, the maximum bilateral difference obtained for the nucleus accumbens was less than 10%, both sides showing the usual TH activation after haloperidol. This attests to the anatomical specificity of the GABA influence on nigral DA neurones, and moreover demonstrates the degree of functional localisation for the material injected.

Data obtained from a series of rats which received intranigral bicuculline suggest that the effects we observed with muscimol are attributable to a specific GABA-mimetic action. Bicuculline methiodide (2.0 nmol per 0.4 μl over 2 min) was injected into the SN 20 min after 0.4 nmol of muscimol (50 ng). Haloperidol (1.0 mg kg^{-1} intraperitoneally) was injected 10 min before bicuculline. Control animals received muscimol and haloperidol, but with saline or strychnine (4.0 nmol per 0.4 μl over 2 min) replaced bicuculline. In all control animals, the apparent K_m of CP–TH for cofactor was 0.8 mM on the muscimol-treated side and 0.3 mM on the contralateral side. In contrast, CP–TH of all bicuculline-treated animals showed no significant bilateral differences: in every case the TH of both neostriata exhibited K_m values (0.25–0.33 mM) characteristic of haloperidol activation. Bicuculline alone had no effect on TH activity, but reversed the muscimol blockade of haloperidol-induced TH activation, and caused the dose–response relationship for muscimol to shift to the right approximately twofold. Since bicuculline (a putative GABA receptor antagonist[19]), but not strychnine (a glycine receptor antagonist[19]), reversed the action of muscimol and reinstated the effect of haloperidol on CP–TH activity, it is likely that GABA receptors mediate the action of muscimol in the SN.

Our data demonstrate that it is possible to interfere with haloperidol-induced activation of CP–TH by pharmacologically maintaining the functional effect of GABA on nigral neurones. It is unlikely that the muscimol–haloperidol interaction occurred directly in the nigra, since in other experiments we found that application of haloperidol (10^{-6}–10^{-9} M) directly into the nigra did not alter CP–TH activity. Thus the release of DA by nigral dendrites[20,21] and a postulated self-inhibitory action of DA in SN[1,22] may not be relevant to the mechanism of neuroleptic activation of CP–TH. Instead, it seems that haloperidol acts to change activity in nigral neurones indirectly, as a result of its action in the neostratium. We suggest therefore that application of muscimol directly into the nigra, by mimicking GABA, acted to preclude the influence of a striatonigral feedback pathway. This implies that a decrease in GABA release in nigra is required for the neuroleptic-induced activation of CP–TH, a contention supported by the ability of bicuculline to reinstate TH activation after muscimol blockade.

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Temperature-dependent inhibition of evoked acetylcholine release in tick paralysis

IN AUSTRALIA, the tick *Ixodes holocyclus* can produce a potentially fatal, flaccid paralysis in various hosts, including man, apparently due to the secretion of a neurotoxin[1,2]. In other parts of the world other species of ticks can produce a similar syndrome[3], but it is not yet clear whether the pathogenesis of the paralysis is the same in all cases. We have found that the paralysis produced by *I. holocyclus* is due to a temperature-dependent inhibition of evoked release of acetylcholine at the neuromuscular junction.

Application of about 10 nymphal *I. holocyclus* produced paralysis in mice after 3.5–4.5 d, the exact period required depending on the rate of feeding of the ticks. Experiments were carried out on phrenic nerve–hemidiaphragm preparations removed from the most severely affected of these animals. These were mounted in a 2-ml bath and perfused continuously, at a rate of 1 ml per min, with modified Krebs' solution (120 mM NaCl, 3.5 mM KCl, 2.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, 11 mM glucose, 25mM $NaHCO_3$) bubbled with 5% CO_2 in O_2. Temperature was monitored by means of a thermistor probe placed close to the recording site. The nerve was stimulated using a suction electrode and conventional intracellular techniques were used to record from the endplate region of muscle fibres. Microelectrodes were filled with 3 M KCl. Compound action potentials were recorded from the phrenic nerve using a second suction electrode. Direct stimulation of the muscle fibres was accomplished by silver wire electrodes placed on either side of the diaphragm.

At first the ability of muscles from paralysed animals to contract in response to nerve stimulation was assessed visually. At room temperature (approximately 23 °C) the muscle always contracted strongly. As the temperature was raised the response declined progressively, and at 37 °C no contraction was apparent. On subsequent reduction of the temperature the muscle response returned. The muscle contracted strongly in response to direct stimulation within this range of temperature. Similar results were obtained from all twenty preparations examined.

The effects of *d*-tubocurarine [*d*-TC] and increased magnesium chloride concentration (with compensation by reducing sodium chloride concentration) on paralysed preparations were also examined. At a concentration of 2.1 μM in the case of *d*-TC or 5 mM in the case of

magnesium chloride, the contractile response of the muscle ceased at a lower temperature than in normal solutions. At temperatures above 30 °C, no twitch occurred. In experiments on preparations from normal mice, the same concentrations of these drugs were insufficient to prevent muscle contraction.

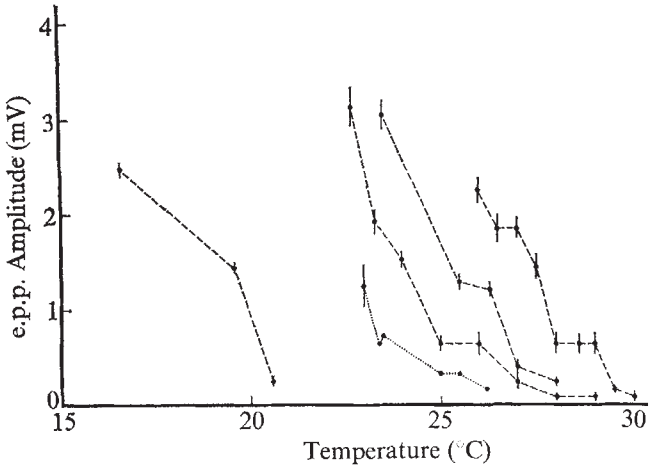

Fig. 1 Graph showing effect of temperature on e.p.p. amplitude at five endplates in three different affected animals. Dashed lines indicate endplates examined in 5 mM Mg^{2+}; dotted line, an endplate examined in 2.1 μM *d*-TC. Each point represents the mean (± s.e.) of approximately twenty responses.

Endplate potentials (e.p.p.s) were examined in these modified solutions. In the temperature range 15–30 °C, muscle fibres could always be found in which nerve stimulation produced e.p.p.s up to 10 mV in amplitude, but none were found above 30 °C. The mean amplitudes of e.p.p.s in muscle fibres from paralysed mice were markedly temperature dependent. As the temperature was increased, the mean amplitude fell and the number of stimuli failing to produce an e.p.p. increased. In each fibre the rate of decline of e.p.p. mean amplitude appeared to be approximately the same, but the temperature range over which the decline occurred differed. This is demonstrated in Fig. 1. The effect could be reversed by lowering the temperature. The amplitude of miniature endplate potentials (m.e.p.p.s) was not altered over this temperature range (Fig. 2).

In preparations from normal mice, the effect of temperature on transmitter release was examined in 8.4 μM *d*-TC or 12 mM Mg. There was no decline in e.p.p. amplitude, comparable with that seen in tick paralysed preparations, over the temperature range 25–37 °C (Fig. 3).

There was no decline in the amplitude of the compound action potentials recorded from phrenic nerves removed from paralysed preparations within this range of temperature. There was, as expected[4], a small increase in amplitude as temperature was increased.

It is unlikely that the paralysis is due to a selective failure of conduction in the nerve terminals. Krnjevic and Miledi[5] have shown that this type of failure can occur, but that it results in a sudden failure of e.p.p.s and is more likely to occur at low temperatures.

Another possibility is that the toxin produces a temperature-dependent, graded reduction of action potential amplitude in the nerve terminals. This possibility has not been entirely eliminated. It seems unlikely, however, that the amplitude of the nerve terminal action potential could be reduced sufficiently to abolish transmitter release without a detectable effect on that of the nerve trunk.

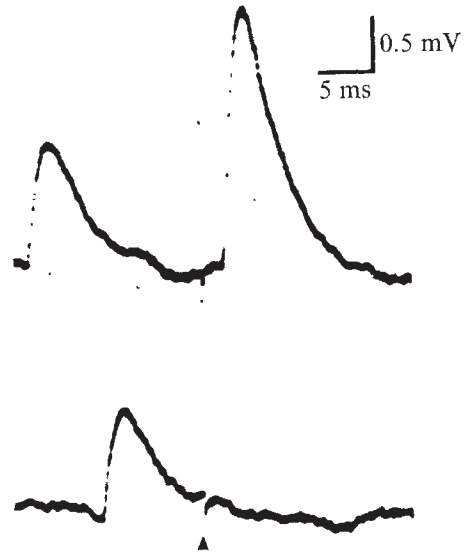

Fig. 2 Endplate potentials and m.e.p.p.s from the same affected endplate at 26.5 °C (upper trace) and 28.5 °C (lower trace). Arrow indicates stimulus artefact.

Other workers[6,7] have suggested that the paralysis produced by the tick *Dermacentor andersoni* is associated with a reduction in amplitude of the compound action potential recorded from peripheral nerves *in vivo*. We found no evidence of this in our experiments and it may be that the modes of action of the toxins from the two species of tick differ. A central action for the toxin of *D. andersoni* has also been postulated[8,9]. Our observations, however, suggest that most aspects of the motor paralysis produced by *I. holocyclus* can be explained on the basis of action at the neuromuscular junction.

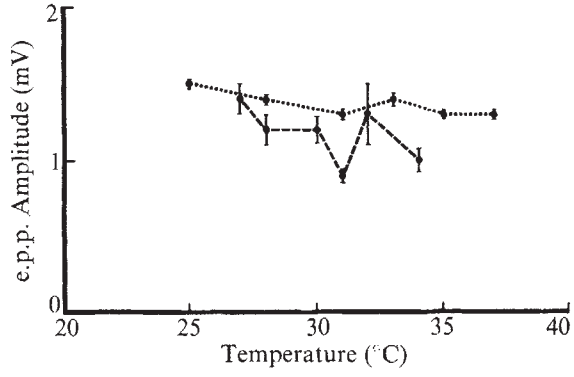

Fig. 3 Graph showing effect of temperature on e.p.p. amplitude in preparations from normal mice. Dashed line indicates an endplate examined in 12 mM Mg^{2+}; dotted line an endplate examined in 8.4 μM *d*-TC. Each point represents the mean (± s.e.) of approximately twenty responses.

The data presented here suggest that the toxin secreted by *I. holocyclus* has a direct, temperature-sensitive action on the excitation–secretion coupling mechanism, which results in an inhibition of transmitter release at the neuromuscular junction. This inhibition seems to be due to a reduction in quantal content, rather than quantal size, as the latter would be expected to result in m.e.p.p.s of reduced amplitude. Because spontaneous release continued when evoked release was blocked, it seems likely that the toxin does not interfere with the actual release mechanism, but rather with some intermediate step between depolarisation of the terminal membrane and release. An attractive

hypothesis is that the toxin blocks the influx of calcium ions which seems to be essential before evoked release can occur[10,11]. This remains to be investigated.

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Mesolimbic dopaminergic neurones in the rotational model of nigrostriatal function

AT least some of the symptoms of Parkinson's disease are believed to result from the degeneration of nigrostriatal dopaminergic neurones which normally innervate the striatum. Parkinsonian patients have an abnormally low concentration of dopamine in this region of the basal ganglia[1,2], and parkinsonian symptoms can be alleviated by the dopamine precursor L-dopa[3]. A quantitative animal model which has proved useful in assessing the potential therapeutic value of drugs in the treatment of parkinsonism was described some years ago by Ungerstedt and Arbuthnott[4]. Rats, in which the nigrostriatal pathway has been destroyed unilaterally by 6-hydroxydopamine (6-OHDA), were shown to rotate towards the lesioned side when injected with drugs, such as amphetamine, which release dopamine from neurones in the brain, and towards the unlesioned side when injected with L-dopa and dopamine agonists[5,6]. This contralateral rotation has been attributed to supersensitivity of the denervated striatal dopamine receptors[5,6]

We have investigated now whether another system of dopaminergic neurones, the mesolimbic system is involved in the drug-induced rotational behaviour. This system, comprised of dopaminergic neurones which originate in the A10 group of cell bodies and which innervate the nucleus accumbens and olfactory tubercle[7], has recently been shown to be important in the locomotor behaviour produced by various drugs[8–15]. In a recent study of the possible role of mesolimbic dopamine neurones in rotational behaviour it was shown that amphetamine and the dopamine agonist apomorphine caused no rotation in rats with unilateral 6-OHDA-induced destruction of mesolimbic dopamine neurones[16]. However, it remained possible that bilateral destruction of the mesolimbic dopamine system could affect the drug-induced rotation of rats with unilateral lesions of the nigrostriatal pathway. We have now found this to be the case.

Adult male Sprague–Dawley albino rats received an intrastriatal injection of 6-OHDA into the right caudate nucleus and during the same operation bilateral injections of 6-OHDA or vehicle into the nucleus accumbens. 6-OHDA injections were made stereotaxically while the rats were anaesthetised with Equithesin (3 ml kg^{-1}). Stereotaxic coordinates for the nucleus accumbens were +3.4, 1.7, 7,2 according to the atlas of Pellegrino and Cushman[17], and for the caudate nucleus were +2.0, 3.0, 5.5. Injections were made through a 30-gauge stainless steel cannula at the rate of 1 μl min^{-1}. Eight micrograms (calculated as base) of 6-hydroxydopamine hydrobromide (Sigma), dissolved in 0.9% saline containing ascorbic acid (1 mg ml^{-1}) was injected in a volume of 2 μl. Vehicle injections were identical except for the omission of 6-OHDA from the solution. At various times following these lesions the rotation induced by intraperitoneal injections of (+)-amphetamine sulphate or the dopamine agonist apomorphine (hydrochloride) was measured. The rats were placed in hemispherical white plastic bowls and the number of turns per min recorded every 10 min by direct observation. Fifty-five to sixty days after the operation the rats were killed by decapitation and their brains removed for the measurement of regional catecholamine content. The brains were chilled on an ice-cold glass plate and the olfactory tubercles, nuclei accumbens, right and left striata, and neocortex were dissected as described previously[18,19]. Tissues were homogenised in 0.1 N perchloric acid containing 0.1 mg ml^{-1} EDTA and assayed for noradrenaline and dopamine by a radioenzymatic method[20] modified from that of Cuello *et al.*[21] The significance of statistical differences was calculated using Student's *t* test.

Fig. 1 shows the dopamine concentration in the nucleus accumbens and striata of the two groups of animals. In both groups the dopamine content of the right striatum was reduced to 35% of that of the left ($P<0.01$). In addition, the 6-OHDA lesion of the nucleus accumbens reduced the dopamine content of the nucleus accumbens to 25% of control ($P<0.01$) and that of the olfactory tubercle to 41% (6.7 ± 0.6 against 2.7 ± 0.4 $\mu g\ g^{-1}$, $P<0.01$). Neocortical noradrenaline content also was reduced to 49% (0.35 ± 0.01 against 0.17 ± 0.01 $\mu g\ g^{-1}$, $P<0.01$) of control by the nucleus accumbens lesion.

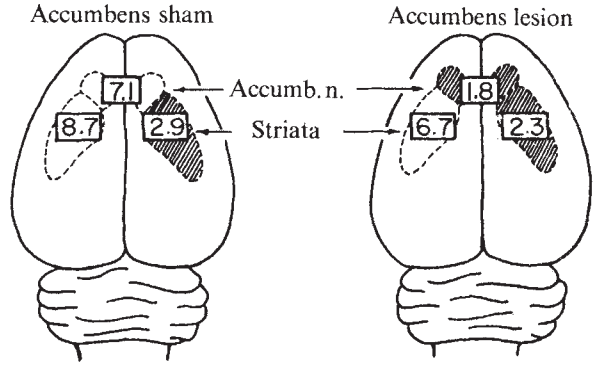

Fig. 1 Diagrammatic representation of the dopamine concentration ($\mu g\ g^{-1}$) in the nucleus accumbens and left and right striata of the two groups of rats (7 rats per group) used in the circling experiments. Shaded areas indicate 6-OHDA (8 μg in 2 μl) was injected into the region.

Figure 2 shows the responses of the two groups of animals to intraperitoneal injections of amphetamine (5 mg kg^{-1}) and apomorphine (0.5 mg kg^{-1}). The effect of the 6-OHDA lesion of the nucleus accumbens was to block the ipsilateral rotation produced by amphetamine, and to enhance the contralateral rotation produced by apomorphine. Although the 6-OHDA lesion of the nucleus accumbens destroyed both mesolimbic dopamine neurons and noradrenergic neurones innervating the neocortex we believe the behavioural effects

of the lesion result from the destruction of mesolimbic dopamine neurones as we have recently obtained similar results when rats were treated with desipramine before the 6-OHDA injection. This pretreatment protects noradrenergic neurones from destruction by 6-OHDA[13,22]. It therefore seems that activity in the mesolimbic dopaminergic system is as important as an imbalance of striatal dopaminergic activity for the expression of drug-induced rotational behaviour. When amphetamine-induced release of dopamine from mesolimbic neurones is prevented by their destruction amphetamine-induced rotation is blocked. The rotation produced by the directly acting dopamine agonist apomorphine, however, is enhanced, presumably because the denervated mesolimbic dopamine receptors become supersensitive.

These findings have implications for the use of rotational models in assessing the effects of drugs as agonists or antagonists at dopamine receptors. First it is clear that the drug-induced rotation of rats with unilateral 6-OHDA-induced destruction of nigrostriatal neurones is not a pure model of striatal dopaminergic mechanisms. Rather it seems that rotational behaviour depends on an imbalance of striatal dopaminergic activity plus activity in the mesolimbic dopamine system. The predominant effect of activity in the mesolimbic system seems to be on the rate of circling rather than its direction. In contrast to the nigrostriatal system there appears to be no directional influence of mesolimbic dopaminergic activity. Rats with unilateral 6-OHDA-induced destruction of mesolimbic dopamine neurones do not rotate in response to amphetamine or apomorphine[16]. Neither do unilateral injections of dopamine agonists into the nucleus accumbens cause any rotation[15].

As a tool for screening potential dopamine agonists and antagonists the rotation model remains useful. It would, however, be wrong to assume that the effects of dopamine agonists and antagonists in this preparation were due solely to their effects on striatal dopaminergic mechanisms. For example, drug-induced rotation would be blocked by drugs which blocked only mesolimbic or only striatal dopamine receptors. This point may be of more than theoretical interest as there is some evidence for differences between mesolimbic and striatal dopamine receptors[24]. The rotation produced by dopamine agonists can clearly be affected by the actions of the drug on mesolimbic dopamine receptors, but our results do not predict whether a drug must act as an agonist at both mesolimbic and nigrostriatal dopamine receptors to produce rotation. It is conceivable, for example, that activation of nigrostriatal dopamine receptors by the drug and release of endogenous dopamine in the mesolimbic system would be sufficient to cause rotation. However, after injections of dopamine or dopaminergic agonists into the striatum the major effect seems to be postural asymmetry rather than a quantitative rotational response[23]. Activation of mesolimbic dopamine receptors by dopamine agonists may therefore be a necessary condition for these drugs to produce rotation.

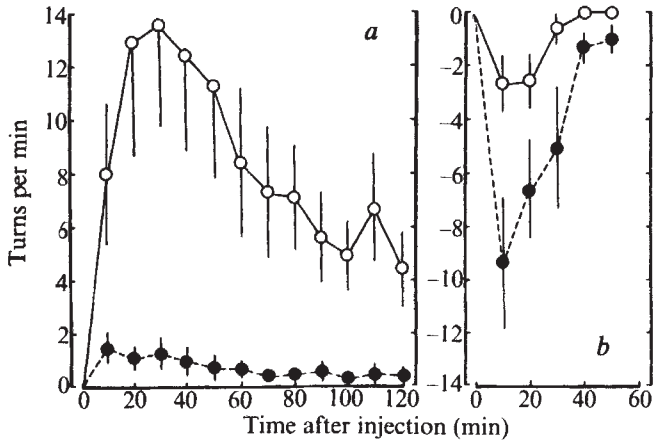

Fig. 2 Turning produced by (+)-amphetamine (*a*) (5 mg kg^{-1}, i.p.) or apomorphine (*b*) (0.5 mg kg^{-1} intraperitoneally) in rats with unilateral 6-hydroxydopamine (6-OHDA) lesions of the caudate nucleus with (●) or without (○) additional bilateral 6-OHDA lesions of the nucleus accumbens. Positive values represent turning towards the lesioned caudate while negative values denote the opposite direction. Mean ±s.e.m. for groups of 7 rats.

Our results show that a drug-induced behavioural expression of nigrostriatal output, rotation, is markedly modified by the concomitant activity at mesolimbic dopamine receptors. Probably, therefore, activity at mesolimbic dopamine receptors can modify the effects of nigrostriatal activity in the undrugged animal, and the mesolimbic and nigrostriatal systems may interact physiologically in the control of motor behaviour. The connections which have been described between the nucleus accumbens and the caudate nucleus[25] and substantia nigra[25–27] might provide an anatomical basis for mesolimbic-nigrostriatal interactions such as those which we have described here. Finally, it is worthy of comment that this and previous work[8–15] emphasises a role of the mesolimbic dopamine system in motor function. Its pathology in motor dysfunction has very recently been reported (O. Hornykiewicz, unpublished).

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Fusion of human erythrocyte ghosts promoted by the combined action of calcium and phosphate ions

Calcium ions are important in almost all membrane fusion systems[1,2]. Murayama and Okada[3] showed that Sendai virus-induced fusion of Ehrlich ascites cells required Ca^{2+}. When

Ehrlich ascites cells were treated with Sendai virus in the presence of EDTA, cells agglutinated and lysed but did not fuse[3,4]. Similarly, Ca^{2+} was required for the fusion of erythrocytes stimulated by chemicals such as lysolecithin[5] or glyceryl monooleate[6]. Ca^{2+} plus the ionophore A23187 (ref. 7) or Ca^{2+} at high *p*H (ref. 8) promoted the fusion of chicken erythrocytes. Fusion of phosphatidylserine-rich liposomes was absolutely dependent on the presence of Ca^{2+}[9]. We describe here the fusion of human erythrocyte ghosts promoted by the combined action of Ca^{2+} and phosphate buffer. Human erythrocyte ghosts, because they lack cytoplasm and are easily filled with small or large molecules[10,11] are useful for the investigation of membrane fusion.

Table 1 shows the effects of various bivalent cations and different buffers on the agglutination and fusion of erythrocyte ghosts. Ca^{2+} plus phosphate buffer was the most effective combination for promoting fusion. Ba^{2+} (Table 1) and Sr^{2+} (not shown) with phosphate buffer caused a moderate amount of fusion, and Ca^{2+} and arsenate buffer caused a very small degree of fusion. Fusion was maximal with Ca^{2+} and phosphate buffer after 15–20 min of incubation at 37 °C but the 'polyghosts' formed were unstable and disintegrated after a further 10–15 min. Ca^{2+}, Mn^{2+}, Zn^{2+} and Ba^{2+} agglutinated ghosts in the presence of phosphate buffer (Table 1). Zn^{2+} and Mn^{2+} in phosphate buffer did not promote fusion although they were active agglutinating agents. There seems to be a close correlation between the formation of a precipitate with phosphate ions and the agglutinating effect of bivalent cations. Mg^{2+}, the only cation tested which does not form a precipitate with phosphate, did not agglutinate ghosts. All bivalent metal ions which formed precipitates with phosphate caused agglutination. Fusion thus seems to be distinct from agglutination, and more specific in its bivalent metal ion requirement. Furthermore, in other experiments not shown here, ghosts agglutinated with Ca^{2+} plus phosphate buffer disaggregated when titrated with acetic acid. The amount of acetic acid required for disaggregation was identical to that required to dissolve the precipitate of calcium phosphate formed in the experimental conditions. In Tricine or Tris buffer plus Ca^{2+} neither agglutination nor fusion was observed. To obtain fusion, it was essential to incubate the ghosts in phosphate buffer and then to add the Ca^{2+}. If the Ca^{2+} and the phosphate buffer were preincubated for 20 min at 37 °C before addition of erythrocyte ghosts, calcium phosphate precipitated. When ghosts were added to such a precipitate, there was some agglutination followed by lysis but no fusion. Calcium phosphate has been implicated in the facilitation of extracellular DNA uptake in eukaryotic cells[12].

Table 2 shows the effects of changing the *p*H of the phosphate buffer on the agglutination and fusion of erythrocyte ghosts in the presence of Ca^{2+}. Erythrocyte ghosts were agglutinated from *p*H 7.0 upwards; and fusion was maximal at *p*H 7.5. There was a direct correlation between the amount of calcium phosphate precipitated, which increased with *p*H, and the extent of agglutination.

Table 1 Agglutination and fusion of human erythrocyte ghosts in presence of bivalent cations and various buffers

Experiment	Buffer	Salt	Concentration (mM)	Agglutination	Fusion
(1)	Phosphate	None			
	Phosphate	$CaCl_2$	2	+++	+++
	Phosphate	$BaCl_2$	2	+	+
	Phosphate	$BaCl_2$	4	++	++
	Phosphate	$MnCl_2$	2	++++	—
	Phosphate	$ZnCl_2$	2	++++	—
	Phosphate	$MgCl_2$	2	—	—
(2)	Phosphate	None		—	—
	Phosphate	$CaCl_2$	2	+++	+++
	Arsenate	None		—	—
	Arsenate	$CaCl_2$	2	—	Trace
	Tricine–NaOH	None		—	—
	Tricine–NaOH	$CaCl_2$	2	—	—
	Tris–HCl	None		—	—
	Tris–HCl	$CaCl_2$	2	—	—

Out-dated human blood type O was obtained from the blood bank of Hadassah Hospital, Jerusalem. Blood aged from 4–8 weeks was used (stored blood). In experiment (1) stored human erythrocytes were washed three times with a solution containing 150 mM KCl and 10 mM sodium phosphate buffer, *p*H 7.4 (isotonic solution) as described before[11]. To prepare ghosts, 2.5 ml of 25% (v/v) erythrocytes in the isotonic medium were dialysed for 2 h against 2 l of hypotonic medium containing 40 mM KCl and 10 mM sodium phosphate buffer, *p*H 7.4. Ghosts were resealed by adding 0.125 ml of a solution containing 2.2 M KCl and 0.02 M $MgSO_4$ and incubating them at 37 °C for 15 min. The ghosts were then washed three times in the isotonic solution. Erythrocyte ghosts were fused as follows. Reaction mixtures contained a final concentration of ghosts equivalent to 1.25% (v/v) of the original erythrocytes in a solution containing 120 mM KCl, 30 mM NaCl and 10 mM sodium phosphate buffer, *p*H 7.4. The experiment was initiated by adding bivalent metal ions as shown in the table and incubating the suspensions at 37 °C for 20 min. In experiment (2) erythrocyte ghosts were prepared as described for experiment (1) except that the phosphate buffer in the isotonic and hypotonic solutions was replaced where indicated by 10 mM Tricine–NaOH buffer, 10 mM Tris–HCl buffer or 10 mM sodium arsenate buffer, *p*H 7.4. Ghosts were fused as described for experiment (1). Agglutination and fusion were estimated by phase contrast microscopy. Agglutination: +, clump of 5–10 cells; ++, clumps of 10–20 cells; +++, clumps of 50–100 cells; ++++, big clumps of 100 cells or more. Fusion: +, fusion of two to four cells, and 5–10% of the ghosts fused; ++, polyghosts derived from 5–10 cells, and 10–20% of ghosts fused; +++, large polyghosts derived from 10–20 cells and 60–80% of ghosts fused.

Table 2 Effect of *p*H on agglutination and fusion of human erythrocyte ghosts by Ca^{2+} plus phosphate

*p*H	Agglutination	Fusion	Remarks
5	—	—	
5.5	—	—	
6	—	—	
6.5	+	—	
7	++	+	Fusion after 60 min
7.5	+++	+++	Fusion after 20 min
8	++++	++	Fused cells disintegrated shortly after fusion
8.5	++++	+	
9	++++	+	

Erythrocyte ghosts were prepared as described in Table 1 for experiment (1). Reaction mixtures contained a final concentration of ghosts equivalent to 1.5% (v/v) erythrocytes in a solution containing 120 mM KCl, 30 mM NaCl, and 10 mM sodium phosphate buffer, at the *p*H indicated. The experiment was initiated by adding $CaCl_2$ at a final concentration of 2 mM and the suspensions were incubated immediately at 37 °C for 20 min. Agglutination and fusion were estimated as described in Table 1.

Table 3 Effect of internal ATP on fusion of human erythrocytes or erythrocyte ghosts

Experiment	Cells	Treatment	Addition during fusion	Agglutination	Fusion
(1)	Fresh human erythrocytes	Stored at 4 °C	None	−	−
			2 mM $CaCl_2$	++++	−
		Incubated at 37 °C with glucose, adenine, inosine and phosphate	None	−	−
			2 mM $CaCl_2$	++++	−
		Incubated at 37 °C with NaF (ATP-depleted)	None	−	−
			2 mM $CaCl_2$	++++	++
(2)	Human erythrocyte ghosts		None	−	−
			2 mM $CaCl_2$	+++	+++
	ATP-containing human erythrocyte ghosts		None	−	−
			2 mM $CaCl_2$	+++	−

In experiment (1), fresh human blood type O was used. ATP-depleted blood was prepared as described before[26]. The erythrocytes were washed in a solution containing 135 mM KCl, 5.4 mM NaCl, 0.8 mM $MgSO_4$ and 20 mM Tricine–NaOH, *p*H 7.4. Blood, 2% (v/v) was incubated overnight at 37 °C in the presence of 10 mM NaF for depletion of ATP or in the presence of 5 mM glucose, 10 mM adenine, 2 mM inosine and 5 mM sodium phosphate as a control. The samples were washed three times in a solution containing 120 mM KCl, 30 mM NaCl and 20 mM Tricine–NaOH, *p*H 7.4. Blood not incubated overnight was washed in the same solution. The three kinds of erythrocytes were suspended in the above solution at a concentration of 1.25% (v/v) in the presence of 2 mM $CaCl_2$ and 5 mM glucose (glucose was omitted in the depleted blood) for 30 min at 37 °C. The samples were then washed three times in a solution containing 120 mM KCl, 30 mM NaCl and 10 mM sodium phosphate buffer, *p*H 7.4, and suspended at a concentration of 2.5% (v/v). For fusion, $CaCl_2$ at a final concentration of 2 mM was added to each of the erythrocyte suspensions and they were transferred immediately to 37 °C for 20 min with slow shaking. Agglutination: ++++, big clumps of more than 100 cells. Fusion: ++, polyerythrocytes made of 10–30 cells and 20–30% of the cells were fused. In experiment (2) stored human erythrocytes type O+ were washed three times in a solution containing 160 mM KCl and 20 mM Tricine–NaOH, *p*H 7.4. Ghosts were prepared as follows: ATP was trapped in human erythrocyte ghosts by dialysis of 1.25 ml of 25% (v/v) of erythrocytes in the presence or absence of 5 mM ATP for 2 h, against 1 l of a hypotonic medium containing 40 mM KCl and 10 mM Tricine–NaOH, *p*H 7.4 at 0 °C. To 1 ml of each system of ghosts 0.050 ml of a solution containing 2.2 M KCl and 0.02 M $MgSO_4$ was added. The ghosts were resealed for 5 min at 37 °C then washed in a solution containing 150 mM KCl and 10 mM sodium phosphate buffer, *p*H 7.4 and suspended at a concentration equivalent to 25% (v/v) of the original erythrocytes. Fusion was achieved as described in Table 1, experiment (1). The cells were incubated at 37 °C for 30 min. Agglutination and fusion were estimated as described in Table 1, experiment (1).

Fresh human erythrocytes incubated with Ca^{2+} and phosphate buffer at *p*H 7.4 agglutinated but did not fuse (Table 3). It seemed possible that the failure of intact erythrocytes to fuse was attributable to the low concentration of internal Ca^{2+} maintained by their Ca^{2+} pump. Since depletion of ATP inhibits the Ca^{2+} pump which expels Ca^{2+} from the erythrocyte cytoplasm[13], the effect of ATP depletion on fusion was tested. Table 3 shows that ATP-depleted erythrocytes fused readily in phosphate buffer plus Ca^{2+}. The polyerythrocytes formed were unstable and disintegrated about 10 min after fusion. The inclusion of ATP in erythrocyte ghosts causes internal Ca^{2+} to be pumped out of them[14], so it was of interest to test the fusion of ghosts containing trapped ATP. Erythrocyte ghosts containing ATP agglutinated, but did not fuse, in conditions where ghosts without ATP fused freely (Table 3). These observations could be explained by assuming that fusion of erythrocytes or their ghosts requires intracellular Ca^{2+}. In earlier experiments, Ahkong *et al.*[7] showed that chicken erythrocytes, pretreated with neuraminidase, fused when Ca^{2+} was introduced to the cytoplasmic side of the membrane with cation ionophore A23187.

We have achieved fusion of human erythrocyte ghosts without the aid of virus, using Ca^{2+} and phosphate ions at a physiological *p*H and temperature. Previously, virus-induced fusion of human erythrocyte ghosts was observed only after gradual haemolysis in the presence of bovine serum albumin[15] or after drastic haemolysis followed by treatment with sulphydryl group-blocking agents[16].

Addition of Ca^{2+} to the medium causes the aggregation of intramembrane particles of human erythrocyte ghosts[17]. Presumably the Ca^{2+} penetrates to the cytoplasmic side of the membrane where it causes polymerisation of spectrin and consequent intramembrane particle aggregation[17]. It has been suggested that fusion of cells involves the rearrangement of intramembrane particles[18]. Because of the apparent interrelationships of Ca^{2+}, intramembrane particle rearrangement and fusion, we used freeze-etching to investigate the distribution of intramembrane particles in ghosts fused with Ca^{2+} and phosphate ions. Figure 1*a* shows a section of erythrocyte ghosts fused with Ca^{2+} and phosphate ions. A polyghost agglutinated with unfused ghosts can be seen. Figure 1*b* shows a freeze-fracture picture of two cells whose membranes are in close juxtaposition. Most of the area of the inner phase of the membrane in both cells contains crowded intramembrane particles. The area of close contact of the two cells (arrow) is interesting in that it is almost devoid of intramembrane particles, and, there seems to be an interdigitation of membrane projections. It seems likely that this is an area of contact just before fusion.

Figure 1*c* is a freeze-fracture picture from a ghost preparation after fusion. The fracture face of the inner phase of the membrane consists of two areas studded with tightly packed intramembrane particles connected by a folded smooth area almost devoid of particles. It is tempting to suggest that the smooth area is a region of fusion joining two cells. No such smooth areas were detected in unfused controls. Since intramembrane particles are probably membrane proteins or glycoproteins, and fusion of cells has been suggested to occur between areas of exposed phospholipids[19], intramembrane particles might have been removed from regions in which fusion occurs. Previous work in our laboratory showed that on clustering of intramembrane particles, phospholipids are exposed and become susceptible to attack by chemical agents and phospholipases[20,21].

There are strong indications that Ca^{2+} is involved in certain biological processes involving membrane fusion such as secretion of macromolecules[22], endocytosis[23], fusion of myoblasts[24], and fusion of Golgi vesicles[25]. It is possible that fusion in these cases occurs by a similar mechanism to that suggested here.

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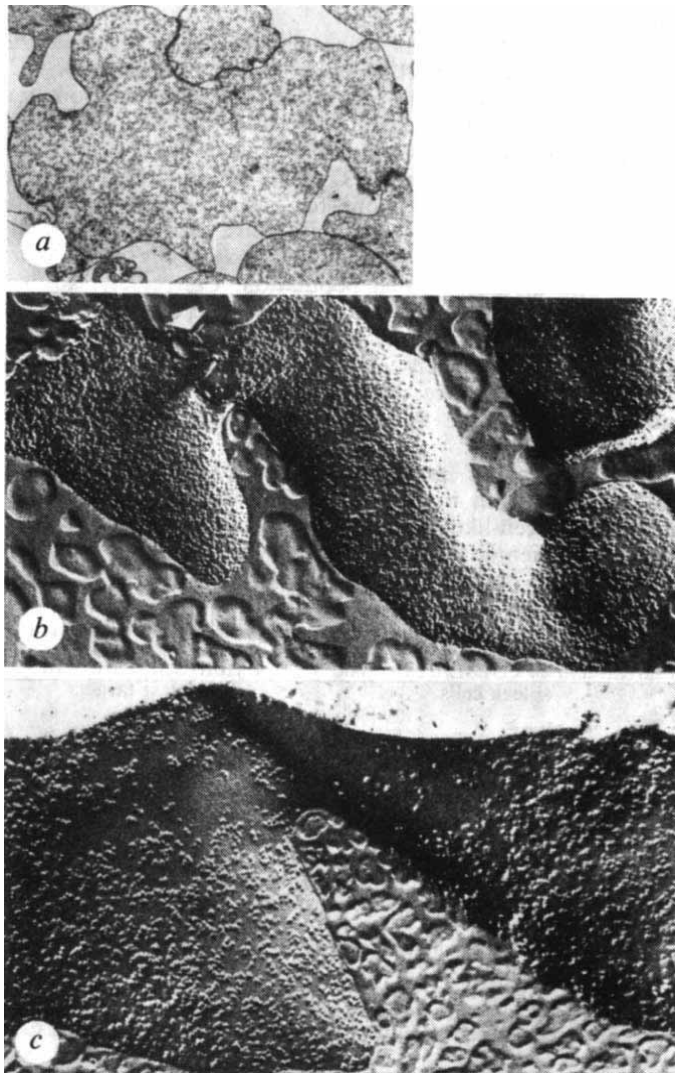

Fig. 1 Electron micrographs of human erythrocyte ghosts in the process of fusing. Human erythrocyte ghosts were prepared and fused as described in the legend to Table 1, experiment (1). The bivalent metal used was $CaCl_2$ (2 mM). Samples were incubated for 25 min at 37 °C for fusion. Preparation of sections for electron microscopy (*a*) was as described before[27]. Freeze-fractured cells were prepared essentially as described before[20] with the following modifications. Erythrocyte ghosts were fixed immediately after fusion by addition of glutaraldehyde (Ladd Research Industries) to a final concentration of 1% (v/v) and centrifuged at 12,000*g* for 5 min. *a*, Electron micrograph of a polyghost tightly agglutinated with unfused ghosts (× 4,800). *b*, Freeze-fracture picture of two cells tightly agglutinated before fusion. Interdigitated region of contact (arrow) is poor in intramembrane particles (× 38,280). *c*, Freeze-fracture picture of fused ghosts. Note folded smooth area separating surfaces with tightly packed intramembrane particles. The smooth area is presumed to be a region of fusion between two ghosts (× 61,200).

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T lymphocytes with promiscuous cytotoxicity

CYTOTOXIC T lymphocytes generated during a unidirectional mixed lymphocyte culture (MLC) lyse target cells which have the antigenic phenotype of the allogeneic stimulating cells. The cytotoxic effect is restricted to cells that bear the same major histocompatibility antigens (H–2 antigens in mice) as the stimulating cells[1]. H–2 restriction of cytotoxicity is also seen when immune T lymphocytes react *in vitro* to cells bearing viral[2], chemical[3], or minor histocompatibility antigens[4] or following autosensitisation against unmodified fibroblasts[5]. Thus, H–2 restriction of cytotoxic lymphocytes has been observed in many circumstances. We now report that cultures of normal spleen cells generate T lymphocytes that damage target cells regardless of their H–2 phenotype.

To investigate the influence of alloantigens on the generation of cytotoxic T lymphocytes, we cultured normal mouse spleen in the presence or absence of allogeneic stimulator spleen cells or fibroblasts. After 5 d culture, the responder lymphocytes were tested against various fibroblast monolayer targets using a modification of a standard microcytotoxicity assay. In this system the number of adherent fibroblasts remaining after 48 h of incubation with the cultured spleen cells was measured by uptake of radioactive ^{51}Cr. As seen in Table 1*a*, C57L (H–2^b) spleen cells, after incubation with irradiated CBA stimulator cells (H–2^k), caused fourfold greater cytotoxicity of B10.Br (H–2^k) than C57BL/6 (H–2^b) fibroblasts. This demonstrated the expected relative H–2 restriction of T-cell cytotoxicity which results from an allogeneic MLC. The presence of allogeneic fibroblasts also induced H–2-restricted cytotoxicity (Table 1*b*).

In contrast, spleen cells cultured alone, without allogeneic stimulator cells, showed a similar high degree of cytotoxicity against target fibroblasts without H–2 restriction (Table 1*c*). In each of 20 experiments, normal spleen cells cultured for 5 d developed promiscuous cytotoxicity that did not discriminate among the H–2 phenotypes of target fibroblasts.

To identify the effector cell in the population of cultured spleen cells, the influence of anti-θ treatment[6] was tested with the results shown in Table 2. The marked decrease of cytotoxic activity after anti-θ+C′ treatment suggests that a T lymphocyte was necessary for the expression of promiscuous cytotoxicity. This conclusion was supported by the finding that the cytotoxic effect was not decreased by filtering the effector cells through nylon wool. Thus, promiscuous cytotoxicity, like H–2 restricted cytotoxicity[7], seems to be a function of T lymphocytes. Cell-free medium

in which effector lymphocytes had been cultured for 48 h showed no cytotoxicity against target fibroblasts when compared with fresh medium. Therefore there was no evidence for a nonspecific cytotoxic factor produced by the lymphocytes.

Experiments were carried out to identify the requirements for induction of promiscuous cytotoxicity. Fresh spleen cells or spleen cells cultured for up to 48 h did not produce this effect. Cells cultured for 3 d had 50% of maximal cytotoxic activity. Peak activity was usually attained after 4–5 d of culture and persisted undiminished for up to 9 d.

Table 1 Induction of H-2-restricted or promiscuous cytotoxicity by culture of normal spleen cells

Induction culture*				Target fibroblasts		
Spleen cells		Allogeneic stimulator cells				
Strain	H-2	Strain	H-2	Strain	H-2	% Cytotoxicity†
a C57L	b	CBA spleen cells	k	C57L	b	27
				B10.BR	k	98
b B10	b	B10A fibroblasts	a	B10	b	3
				B10.A	a	83
B10.A	a	B10 fibroblasts	b	B10.A	a	8
				B10	b	84
				B10.D2	d	7
				B10.BR	k	5
c C57L	b	None		C57L	b	75
				B10.BR	k	67
B10	b	None		B10	b	92
				B10.D2	d	91
				B10.BR	k	89
				B10.A	a	96
B10.A	a	None		B10.A	a	93
				B10	b	94
				B10.D2	d	94
				B10.BR	k	94
NZB	d	None		NZB	d	78
				B10.A	a	73
C57BL/6	b	None		C57BL/6	b	76
				NZB	d	81
BALB/c	d	None		DBA/2	d	90
				C57BL/6	b	92

*Suspension of spleen cells of 6–8-week-old male mice were prepared by forcing fragments of spleen through a tantalum gauze mesh. The spleen cells were suspended in medium consisting of RPMI 1640 (Grand Island, Gibco) plus 10% foetal calf serum (FCS) (heat inactivated at 56% for 30 min; Gibco), 5×10^{-5} M 2-mercaptoethanol, sodium pyruvate 1 mM (Microbiological Associates), non-essential amino acids 0.1 mM (Microbiological Associates) and penicillin (5,000 units ml^{-1}) and streptomycin (5,000 μg ml^{-1}) (RPMI+10% FCS). The spleen cells were washed, centrifuged and resuspended at a concentration of 7×10^6 viable nucleated cells in 2 ml of RPMI+10% FCS. The spleen cells were incubated for 5 d in 16-mm tissue culture wells (Costar) in a volume of 2 ml per well at 37 °C in moist air plus 10% CO_2. After induction the spleen cells were collected from the culture wells by repeated pipetting of the medium, washed by centrifugation and tested for cytotoxicity. Some induction cultures contained allogeneic stimulator cells which were either 3.5×10^6 irradiated (2,000 r.) spleen cells (*a*), or monolayers of 10^5 fibroblasts (*b*). The fibroblasts were prepared from 13–17-d-old mouse embryos as described[15].

†Cytotoxicity was assayed in quadruplicate by incubating test spleen cells with target fibroblasts at effector–target ratios of either 50:1 or 100:1. Control cultures contained fresh uncultured spleen cells of the same genotypes as the test spleen cells. One million spleen cells in 2 ml RPMI+10% FCS were added to either 10^4 or 2×10^4 target fibroblasts which had been cultured for one day in 16-mm culture wells. After 44 h, the spleen cells and detached fibroblasts were aspirated and the adherent fibroblasts washed four times with RPMI×10% FCS. To measure the relative numbers of adherent fibroblasts, we incubated each culture well for 40 min with 3 μCi ^{51}Cr in 0.2 ml of 0.3 M sucrose. The wells were then washed twice with RPMI+10% FCS and aspirated dry. Uptake of ^{51}Cr was determined by digesting the fibroblasts with 1 ml 0.1 N NaOH. After 30 min the contents of each well were transferred to plastic tubes and radioactivity was measured in gamma counter. Percentage cytotoxicity was computed as (1–mean c.p.m. (target with test spleen cells/target with control fresh spleen cells)×100). The standard deviations were always less than 10% of the mean c.p.m.

Adherent cells such as macrophages are involved in the induction of cytotoxic T lymphocytes by allogeneic cells[8]. The role of an adherent population in the induction and effector phases of promiscuous cytotoxicity was investigated with the results shown in Table 3. Spleen cells were plated on Petri dishes for 1 h at 37 °C. The non-adherent cells were removed, cultured alone for 5 d and then assayed for cytotoxicity. The non-adherent cells had a markedly decreased capacity to generate cytotoxic cells when compared with an unseparated population. Conversely, absorption of spleen cells on Petri dishes after 5 d of culture did not reduce their capacity to mediate cytotoxicity. Fractionation of spleen cells on nylon wool columns before or after *in vitro* culture confirmed the requirement for an adherent cell in the induction of cytotoxicity, but not in the effector phase.

The inducer of promiscuous cytotoxicity is unknown. Foetal calf serum in the culture medium might have contained mitogen-like substances capable of inducing polyclonal differentiation of effector lymphocytes[9]. It is also possible that the foetal calf serum was specifically immunogenic during induction. Its association with the fibroblasts in the target cultures could therefore have triggered cytotoxicity to the fibroblasts[10]. This is unlikely, however, since we found that adding either allogeneic or syngeneic fibroblasts to the inducing cultures led to H-2 restriction of the resulting cytotoxicity (our unpublished results).

Table 2 Effect of anti-θ treatment on promiscuous cytotoxicity

Treatment of C57BL/6 cytotoxic spleen cells	% Cytotoxicity of C57BL/6 fibroblast targets
None	94
C′	91
Anti-θ	93
Anti-θ+C′	33

Promiscuous cytotoxicity was induced and tested as in Table 1. After induction, the spleen cells were untreated, or treated with complement (C) anti-θ globulin or anti-θ globulin+C′ and tested for cytotoxicity against syngeneic fibroblasts (see Table 1). Anti-θ globulin (Cohn Fr. II of AKR anti C3Hθ serum) was added to spleen cells (30×10^6–50×10^6) at a dilution of 1:10 in 0.3 ml and incubated at room temperature for 45 min. C′ (fresh guinea pig serum absorbed with C57BL/6 spleen cells for one hour at 0 °C) was used at a final dilution of 1:9 in 1 ml and incubated at 37 °C for 1 h. Some spleen cells were treated sequentially with anti-θ globulin followed by C′.

Regardless of the underlying mechanism, it is clear that *in vitro* culture of spleen cells can lead to the generation of T lymphocytes with relatively unrestricted cytotoxicity. How then does addition of allogeneic cells to the culture abort promiscuous cytotoxicity? It is possible that the relevant alloantigens may favour the selective proliferation of clones. Alternatively, the induction of specific cytotoxicity by allogeneic cells may be accompanied by the active suppression of unselected clones.

The emergence of nonspecific suppressor activity following *in vitro* culture of normal spleen cells has been observed by Hodes and Hathcock[11] and by Burns *et al.*[12]. "Precultured" spleen cells could not be induced into cytotoxic effectors in an MLC. Such cells also suppressed the responses of fresh spleen cells to allogeneic or modified syngeneic targets[11]. The induction of antibody production *in vitro* was also inhibited by spleen cells cultured in a similar manner[12]. This suppression was mediated by a T lymphocyte and had similar kinetics and cellular requirements for induction as the promiscuous cytotoxicity reported here.

What is the relationship between promiscuous cytotoxicity and the reports of suppressor activity that developed spontaneously in cultures of mouse spleen cells? These

Table 3 Effect of removal of adherent cells on promiscuous cytotoxicity

Absorption of BALB/c spleen cells on Petri dishes	% Cytotoxicity of NZB fibroblast targets
None	76
Before induction	18
After induction	70

Promiscuous cytotoxicity was induced and tested as in Table 1. Some of the spleen cells were absorbed before induction by placing 20×10^6 cells in 3 ml RPMI+10% FCS in 60-mm tissue culture dishes (Falcon) and incubating at 37 °C for 1 h. Non-adherent cells were removed by repeated pipetting, cultured for 5 d and tested for cytotoxicity. Some spleen cells were cultured for 5 d and then absorbed as above. The non-adherent cells were then tested for cytotoxicity.

phenomena conceivably result from activities of distinct subclasses of T lymphocytes: cytotoxic and suppressor cells. Alternatively, these phenomena may reflect the dual function of a single subclass of lymphocytes. In other words, these different *in vitro* measurements may be manifestations of the same biological function. Suppression of an MLC or antibody production[11,12] could result from indiscriminate damage to the reacting lymphocytes. And the effect we observed may be yet another manifestation of suppressor cell function. Identity of suppressor and cytotoxic T lymphocytes is also supported by the finding that the cells mediating these activities share the same Ly-2,3 T-cell marker[13,14].

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New principle for the analysis of chemical carcinogenesis

THE development of cancer following exposure to chemical carcinogens or to various forms of irradiation is almost invariably slow and prolonged. Although the process can be initiated by a brief exposure to a carcinogenic stimulus, there is no evidence that target cells so altered are cancer cells. Rather, there is abundant indirect evidence from many systems that what is induced is an altered cell or cell population from which malignant neoplasia can gradually develop or evolve[1,2]. Neoplastic development therefore resembles a chain reaction, triggered by exposure to a carcinogen, in which the links are new populations with altered organisational, structural and biochemical properties. These slowly proliferative new lesions are characteristically focal in distribution, implying that only a small proportion of the original target cell population in any organ or tissue participates. It is not known what the critical property (or properties) is that makes initiated cells so important in carcinogens and the failure to understand and manipulate this early step has been a major impediment to its analysis.

We have developed a new approach to the study of chemical carcinogenesis which seems to offer the first quantitative assay for populations of initiated cells, as well as the possibility of a sequential analysis of some of the early putative preneoplastic and premalignant hepatocytes for liver cancer. The principle is functional rather than morphological or biochemical and depends on the increasing evidence that the selective growth of focal precancerous liver cells seems to result from their relative resistance to the cytotoxic action of hepatocarcinogens, coupled with the creation of a local environment for their proliferation.

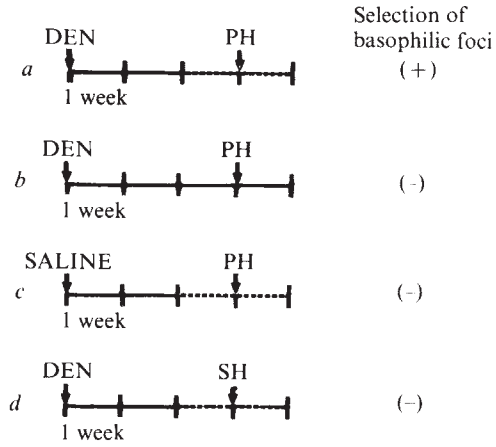

Fig. 1 Schematic representation of the assay procedure (*a*) for initiated cells in liver induced by a carcinogen. *b*, *c*, *d*, Essential controls. DEN, 200 mg per kg body weight intraperitoneally; —, 1 week of basal diet; - - -, 1 week basal diet plus 0.02% AAF; PH, partial hepatectomy; SH, sham hepatectomy.

All known hepatocarcinogens inhibit regeneration of liver cells following partial hepatectomy and at higher doses may lead to liver-cell death. The focal, slowly proliferative cell populations called hyperplastic nodules, that occur in the liver before the appearance of cancer, all acquire new properties relating to the metabolism and toxic effects of hepatocarcinogens and other hepatotoxins that require metabolic activation. These include resistance to hepatotoxin-induced cell death[3], decreased uptake and/or activation of some carcinogens[3], a uniform decrease in several components of microsomal mixed function monooxygenase system including cytochrome *P*450, aryl hydrocarbon hydroxylase and aminopyrine demethylase[4,5] and also the capacity for proliferation following partial hepatectomy during carcinogen administration[6,7].

In the light of these considerations, we wondered

whether an early, or even first, change induced by an hepatocarcinogen might be the induction of an altered metabolic pattern in some liver cells (similar to that seen in hyperplastic nodules), such that these cells would have a potential growth advantage over the majority of original hepatocytes in a cytotoxic environment. If this were the case, it should be possible to detect the altered cells soon after initiation by creating an intense stimulus for proliferation in the presence of a toxic growth inhibitor requiring metabolic activation. Initiated cells with a decreased capacity for activation might proliferate rapidly, whereas cells unaltered in this respect would activate the carcinogen and thereby generate a derivative which would inhibit their proliferation. On the basis of this theory, we devised an assay system for such resistant cells (Fig. 1*a*).

The assay system consists of three components: an initiator, for example, diethylnitrosamine (DEN); a selective growth inhibitor, 2-acetylaminofluorene (2-AAF), and a generalised potent growth stimulus, in this case, partial hepatectomy (PH). The major carcinogen so far studied has been DEN, since it can induce liver cancer in the rat slowly with a single oral, intravenous, or intraperitoneal administration[8,9]. DEN was given intraperitoneally to Fischer-344 rats at a dose of 200 mg kg^{-1}. Following a 2-week period of recovery from the initial cell damage, the animals were fed a standard basal diet (24% protein) containing 0.02% 2-AAF for 1 week and were then subjected to 67% partial hepatectomy. With only 2-AAF and partial hepatectomy, there was no hepatocyte proliferation, and no labelling of hepatocyte nuclei with ^{3}H-thymidine as observed autoradiographically. In animals pretreated with DEN, however, there were multiple focal islands of proliferating intensely basophilic hepatocytes (Fig. 2) distributed randomly throughout the liver which started to appear about 30 h after operation. They grew rapidly, so that within 7–10 d they are visible grossly as small, greyish-white nodules about 1 mm in diameter. No such foci were seen in animals treated with saline in place of DEN, given 2-AAF without PH, subjected to PH without concomitant exposure to 2-AAF or subjected to sham operation. Although subsequent studies have shown that feeding the 2-AAF diet for about 3 weeks before PH is sufficient to induce a few basophilic foci, they were rarely seen in the present experiments without pretreatment with DEN.

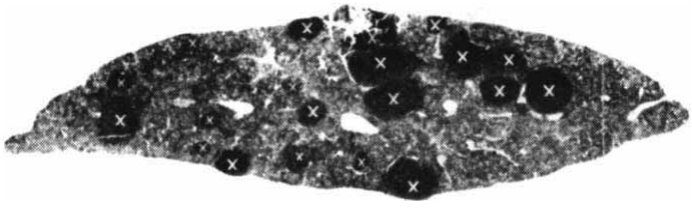

Fig. 2 Low power view (×7) of a routine histological section of liver from a rat showing many initiated basophilic foci (X). This animal received 200 mg kg^{-1} DEN according to the regimen in Fig. 1*a* and was killed 9 d after partial hepatectomy.

The number of foci induced by DEN varies with the dose. Figure 3 shows the relative 'focus-forming ability' over a dose range of 20 to 200 mg kg^{-1}. The foci could be counted either by low power examination of histological sections (Fig. 2) or at 7–10 d by counting the foci in a gross liver section, the sections of liver having been taken from a standard site. Foci similar to those seen with DEN have been induced by every hepatocarcinogen studied to date, including dimethylnitrosamine, aflatoxin B1, and *N*-hydroxy-2-AAF.

The initiated foci can be labelled with ^{3}H-thymidine without labelling the surrounding original hepatocytes and in this way, the fate of the hepatocytes in the foci can be followed. If the dietary 2-AAF is discontinued following partial hepatectomy, selective proliferation of the foci stops as the majority of original hepatocytes recover the ability to proliferate. By manipulating the conditions, it thus becomes possible to control the number of cell proliferations and to study their role in the development of cancer.

Following termination of the specialised regimen at 7 d after operation, the foci continue to grow, so that by 8 weeks the liver is composed of many hyperplastic nodules indistinguishable from those described previously[10,11]. If the animal is not subjected to the 'selection pressure' used (2-AAF plus PH), the latent or potential foci persist for at least 10 weeks without any significant change in size or number. Thus, the initiated hepatocytes induced by DEN have not yet acquired the property of autonomous growth but can be induced to grow in an appropriate environment. Also, we have obtained no evidence for their recognition by the host or for their removal or repair. The later development of some autonomous growth of such cells represents another step in the sequence before the ultimate development of malignant neoplastic cells.

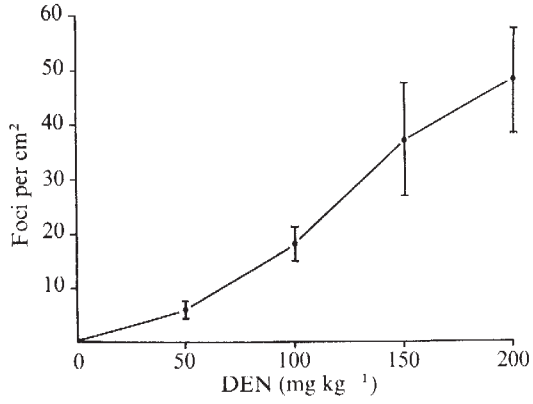

Fig. 3 Induction of foci of initiated liver cells as a function of the dose of diethylnitrosamine (DEN). The number of foci was counted in sections from each lobe by low power microscopy and is expressed per cm^2 of cross-sectional area (mean ± s.e.m.).

Scherer and Emmelot detected histochemically and counted microscopic foci of enzyme-deficient hepatocytes induced with DEN[12]. These foci may prove to be the initiated populations selected in the present system.

The interval between the administration of carcinogen and the assay is being shortened so that the kinetics of induction of the initiated foci can be established clearly with different carcinogens. Another desirable modification would be the use of a non-carcinogen as the selective growth inhibitor. Even in its present form, however, the assay is useful for the study of several aspects of chemical carcinogenesis, including the possible role of DNA synthesis and of repair of specific DNA damage in initiation, the persistence of initiated cells, the sequential analysis of the properties of hepatocytes acquired after initiation, the possible functional theme underlying the development to liver cancer and the clarification of some confusing aspects in the use of liver hepatoma formation in mice as an assay for carcinogens.

The hypothesis of differential cytotoxicity as an important theme for chemical carcinogenesis is not new[4,10,13–17], but it has never been subjected to critical tests. On the basis of our results, it is tempting to consider that the acquisition of resistance to some of the toxic effects of an added carcinogen or, in the case of a single exposure to a carcinogen, to toxic xenobiotic compounds in the natural

diet or absorbed bacterial products in the intestinal tract, may be the major motive force during liver carcinogenesis until the precancerous cells can grow autonomously. Consistent with this view of liver carcinogenesis are the findings of Peraino and colleagues on the acceleration of liver carcinogenesis by phenobarbital or DDT in animals exposed for only 18 d to 2-AAF (refs 18, 19). An underlying hypothesis for liver carcinogenesis is now developing, which is amenable to experimental tests. Whether the principle of selective cytotoxicity can be extended to the development of cancer in other organs or tissues remains to be explored.

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Ammonia assimilation in lupin nodules

ROBERTSON *et al.*[1,2] proposed that plant enzymes rather than bacteroid enzymes are physiologically important in the assimilation of the ammonia produced after nitrogen reduction in the bacteroids of leguminous nodules. They demonstrated that during the development of the nodule, glutamine synthetase (EC 6.3.1.2, equation (1)) and glutamate synthase (EC 2.6.1.53, equation (2)) activity increased considerably in the plant fraction but not in the bacteroid fraction of the nodule, parallel with the induction of nitrogenase and leghaemoglobin.

$$\text{glutamate} + NH_4^+ + \text{ATP} \longrightarrow \text{glutamine} + \text{ADP} + P_i \quad (1)$$

$$\text{glutamine} + \text{2-oxoglutarate} + \text{NADH} \longrightarrow 2\ \text{glutamate} + NAD^+ \quad (2)$$

But, the predominant amino acid transported in the xylem of lupins and many other legumes is asparagine[2–4]. Robertson *et al.*[2] suggested therefore that asparagine was most probably synthesised in the plant fraction of the nodule by a glutamine-dependent asparagine synthetase[5,6] (EC 6.3.5.4, equation (3)) rather than in the bacteroids, which might utilise for asparagine biosynthesis an ammonium-dependent asparagine synthetase (EC 6.3.1.1, equation (4)), which has been reported to occur in other prokaryotes[7].

$$\text{aspartate} + \text{glutamine} + \text{ATP} \longrightarrow \text{asparagine} + \text{glutamate} + \text{AMP} + PP_i \quad (3)$$

$$\text{aspartate} + NH_4^+ + \text{ATP} \longrightarrow \text{asparagine} + \text{AMP} + PP_i \quad (4)$$

We now report the formation of asparagine by a cell-free extract of the plant fraction of lupin nodules in the presence of ATP, aspartate, glutamine and Mg^{2+} (Table 1). Apparent K_m values for aspartate and glutamine were determined to be 3.6 mM and 0.26 mM, respectively. Ammonia could replace glutamine as the source of the amide nitrogen; but a fourfold greater concentration of ammonia gave only a rate of synthesis half that observed with glutamine (Table 1). Asparagine synthetase activity could not be detected unless the nodules were crushed in the presence of a sulphydryl protecting agent such as dithiothreitol. Furthermore, the enzyme was found to be stabilised by inclusion of 5 mM glutamine or 6 mM 5-diazo-4-oxo-L-norvaline (DONV), a glutamine analogue in the buffer. These properties of the asparagine synthetase from the plant fraction of *Lupinus angustifolius* nodules are in good agreement with the properties of the enzyme recently purified from etiolated *Lupinus luteus* cotyledons[8].

Asparagine synthetase activity in the plant fraction of the developing nodules was first detected 13 d after the seedlings were inoculated with rhizobia (Fig. 1). The rapid increase in activity seen after day 13 is similar to that already reported for the plant glutamine synthetase (ref. 1 and repeated here Fig. 1), plant glutamate synthase[2], nitrogenase[1] and leghaemoglobin[1]. No asparagine synthetase activity could be detected in the plant fraction of 11 or 12-d-old nodules (Fig. 1). But these fractions contained an asparaginase activity whereas the plant fractions from 13-and 24-d-old nodules did not (Fig. 1). Attempts to detect asparagine synthetase activity in the plant fraction of 11-and 12-d-old nodules in which the asparaginase was inhibited 80–90% with DONV (a known inhibitor of bacterial asparaginases[9]) were unsuccessful (Fig. 1).

Table 1 Synthesis of asparagine by extracts of the plant fraction of lupin nodules

Incubation mixture	Asparagine synthetase activity (nmol asparagine per min per mg protein)
Complete system	2.33
−glutamine	0.30
−glutamine + 13.2 mM NH_4Cl	1.23
$-Mg^{2+}$	ND
−ATP	ND
Boiled enzyme	ND

Lupins (*Lupinus angustifolius* L. var. Uniwhite) were inoculated with *Rhizobium lupini* (NZP 2257) and grown in a controlled environment cabinet[2]. Nodules were collected from 25-d-old plants, crushed in 0.5 M sucrose, 50 mM K phosphate buffer, *p*H 7.8, containing 5 mM dithiothreitol and separated into a bacteroid and plant fraction as previously described[2]. Asparagine synthetase activity was measured at 30 °C by the formation of ^{14}C-asparagine in a total volume of 300 µl containing plant fraction (200 µl), 4 mM L-4-^{14}C-aspartate (0.83 µCi µmol^{-1}), 3.3 mM $MgCl_2$, 3.3 mM L-glutamine and 4 mM ATP. Samples of 25 µl were removed during 15 min of incubation and applied to a Whatman 3 MM paper. Asparagine was separated from aspartate by electrophoresis at *p*H 5.0 and the radioactivity in each amino acid was determined[1]. Identification of the product as asparagine was confirmed by electrophoresis and chromatography using two procedures: (1) electrophoresis in formic acid–water (4% v/v) adjusted to *p*H 2.2 with pyridine (50 V cm^{-1} for 2 h) followed by descending chromatography in *n*-butanol–*n*-butyl acetate–acetic acid–water (19:1:5:25 v/v) and (2) electrophoresis in 0.04 M sodium acetate, *p*H 5.0, (30 V cm^{-1} for 30 min) followed by descending chromatography in methanol–pyridine–water (80:4:20 v/v). Furthermore, the ^{14}C-asparagine eluted from these chromatograms was converted to ^{14}C-aspartic acid by hydrolysis in 2 M HCl at 105 °C for 16 h.

ND, not detectable, less than 0.2 nmol per min per mg of protein.

The decrease in asparaginase activity in the plant fraction at days 11 and 12 coupled with the complete absence of this enzyme on day 13 (Fig. 1) was particularly interesting. This plant asparaginase was different from the bacteroid or free-living rhizobial enzyme in terms of (1) electrophoretic mobility on 7.5% polyacrylamide gels relative to bromophenol blue (0.47 for the plant enzyme and 0.27 for the bacteroid or rhizobial enzyme) and (2) apparent K_m for asparagine (7 mM for the plant enzyme and 6 μM for the rhizobial enzyme). In view of the fact that the plants were grown in a nitrogen-free medium, it seemed likely that until the bacteroids developed nitrogen-fixing ability, the plant asparaginase had a role in supplying the developing nodule tissue and bacteroids with ammonia by hydrolysing asparagine transported from the cotyledons. Once nitrogen fixation had been established 11–12 d after inoculation the plant asparaginase would no longer be required and activity would be lost (Fig. 2). An analogous role for a plant asparaginase in supplying rapidly developing legume seed tissue with C and N has been proposed by Atkins *et al.*[10].

So far we have been unable to detect any ammonium- or glutamine-dependent asparagine synthetase activity in either the bacteroid fraction of the nodules or in cell-free extracts of the free-living rhizobia grown in a broth or minimal medium. Attempts were made to inhibit or remove an asparaginase activity from these extracts before assaying for asparagine synthetase, but in no case could any synthetase activity be detected.

Our studies reported here and elsewhere[1,2] have led us to suggest that plant enzymes rather than bacteroid enzymes are involved in the assimilation of the ammonia produced from nitrogen reduction in the bacteroid. The first step in this assimilation (Fig. 2) is proposed to be the synthesis of glutamine by the plant glutamine synthetase. It has been estimated previously[2] that the levels of this enzyme found in the plant fraction of the nodule would be able to incorporate into glutamine more than twice the amount of ammonia actually produced by the bacteroids in 18-d-old lupin nodules. On the other hand,

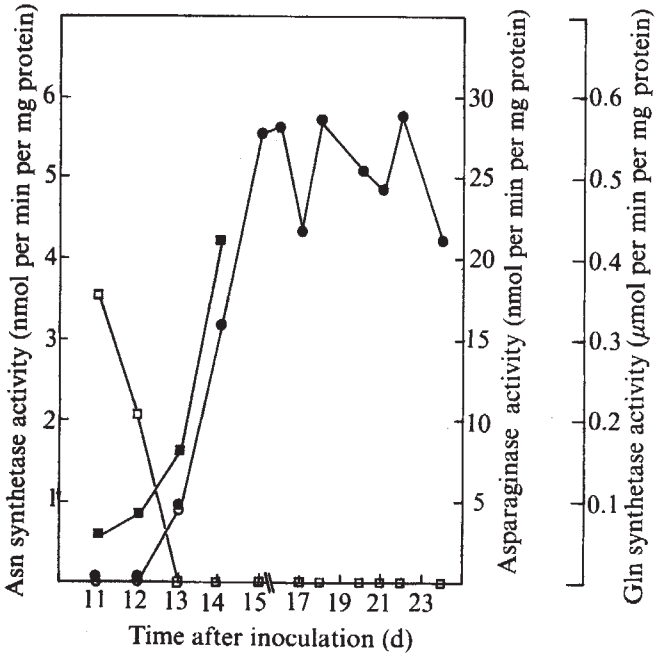

Fig. 1 Levels of asparagine synthetase, asparaginase and glutamine synthetase during nodule development in lupin. Nodules were collected from plants collected at intervals after inoculation and the plant enzyme fraction was prepared as in Table 1. Asparagine synthetase activity was assayed as for Table 1 (●) or after DONV treatment[2] (○). Glutamine synthetase levels (■) were determined as previously described[1]. Asparaginase activity (□) was determined radiochemically using the procedure outlined for glutaminase assays[1], except that ^{14}C-U-asparagine was used as substrate instead of glutamine.

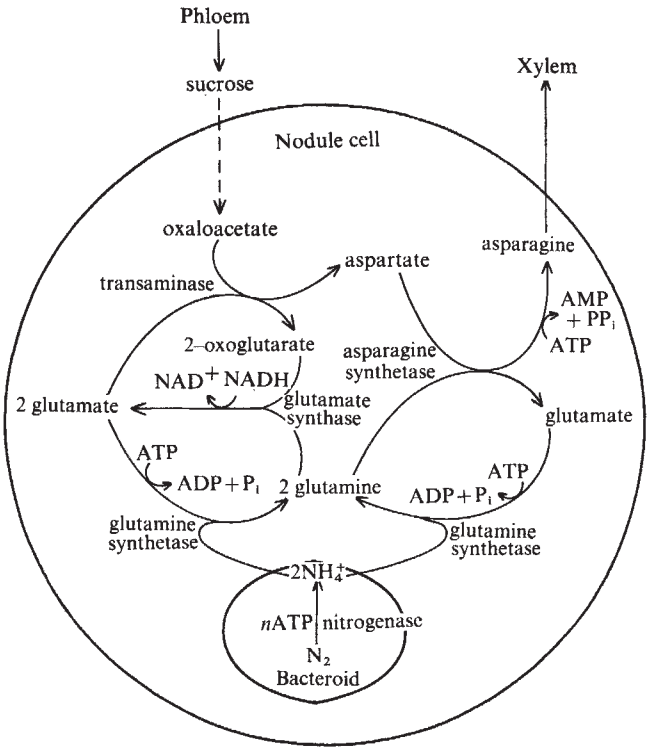

Fig. 2 Proposed pathway for the assimilation of the ammonia produced after nitrogen reduction in the bacteroids into asparagine by enzymes located in the plant fraction of lupin nodules.

less than 3% of this ammonia could be assimilated by the bacteroid enzyme[2], a conclusion recently confirmed by the work of Kurz *et al.*[11] and Brown and Dilworth[12]. The high level of this plant glutamine synthetase, reported here in lupin and in other legume nodules[12], coupled with the low K_m for NH_4^+ (0.02 mM)[13] would provide an efficient mechanism for rapidly removing any ammonia passing out of the bacteroid into the plant. In the presence of this enzyme it is unlikely therefore that the plant asparagine synthetase would be able to incorporate ammonia directly into asparagine since the K_m for NH_4^+ for the plant asparagine synthetase is 3–5 mM (refs 5 and 6).

After the incorporation of ammonia into glutamine in the plant fraction of the nodule tissue, the amide nitrogen of glutamine would according to our model be transferred through glutamate using glutamate synthase and a transaminase to oxaloacetate to give the α-amino of aspartate (Fig. 2). The synthesis of asparagine by the glutamine-dependent asparagine synthetase would then be accomplished using this aspartate and a second amide nitrogen from glutamine (Fig. 2). The net result of these reactions would be the synthesis of asparagine from oxaloacetate and 2 mol of ammonium requiring NADH and 3 ATP.

The role of asparagine as the major amino acid carrier of fixed nitrogen is supported by an examination of the amino acid

Table 2 Amino acid analysis of xylem sap from 11- and 18-d-old lupin plants

Amino acid*	μmol amino acid per ml per sap 11 d	18 d
Asparagine†	0.77	2.35
Aspartate	0.32	0.54
Glutamine†	0.23	0.41
Glutamate	0.33	0.36
Threonine	0.15	0.40

Xylem sap was collected using a pressure chamber[14] and stored under liquid nitrogen. Amino acid analyses were carried out using a JEOL 6A-H analyser.

*The level of all other amino acids was less than 0.1 μmol per ml of sap.

†Determined as the increase in aspartate or glutamate on hydrolysis for 4 h at 105 °C in 1 M HCl.

levels in the xylem sap of 11- and 18-d-old plants (Table 2). Although asparagine is the predominant amino acid transported in both cases, it is the asparagine level which increases and not that of glutamine as the capability of the nodule to fix nitrogen increases. Transpiration rates determined using 18-d-old plants were such that the plants would be transporting asparagine at a rate of 2 μmol per h per plant. Furthermore, we can estimate from the activity of asparagine synthetase reported here, that the nodules could synthesise up to 0.8 μmol asparagine per h per plant, which is in reasonable agreement with the rate of transportation of this amide considering the instability of asparagine synthetase *in vitro*.

In conclusion, we have proposed a pathway by which ammonia produced by the bacteroids is assimilated by the plant fraction of lupin nodules. Presumably the bacteroid enzyme systems provide the ATP and electrons for N_2-reduction whereas the plant enzyme systems provide the additional NADH and ATP required for the incorporation of ammonia into asparagine. Further interest will undoubtedly lie in elucidating the mechanisms regulating the synthesis of nitrogenase in the bacteroids, and leghaemoglobin, glutamine synthetase, glutamate synthase and asparagine synthetase in the plant fraction of the nodule. The regulation of the plant asparaginase activity and the reason why the bacteroids overproduce ammonia for plant growth beyond the level required for bacterial growth are also intriguing questions demanding further study.

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Submarine pollination in seagrasses

THERE is great interest in water plants and their adaptation to the aquatic environment. Seagrasses, in the family Cymodoceaceae, are the only wholly marine group of flowering plants which carry out their entire life cycle in the sea[1]. The male and female flowers are borne on different plants. Consequently, their pollination mechanism presents intriguing problems, for terrestrial angiosperms, from which seagrasses presumably arose[2,3], shed dry pollen. This becomes hydrated only after alighting on the female stigma, where recognition events determine acceptance or rejection of the pollen[4]. These events seem to involve interactions between surface proteins or glycoproteins borne by both pollen and stigma[5]. The pollen proteins are released on to the stigma during normal pollination[6,7], but are lost from the surface whenever pollen is wetted[8]. The system found in terrestrial species could thus hardly operate with submarine pollination. We have found that adaptations have occurred in both pollen and stigma of the seagrasses, accommodating the pollination system to the marine environment. Changes in the shape and form of the pollens, together with the loss of the outer wall layer, make it possible for the pollen to be carried in water currents as long, rope-like masses. The receptive stigma cells secrete a proteinaceous surface layer that is not dispersed in seawater, providing a suitable medium for trapping the pollen during submarine pollination.

We have used two species of sea nymph, *Amphibolis antarctica* and *A. griffithii*, found only on the temperate southern coast of Australia, and *Thalassodendron ciliatum*, widely distributed in tropical parts of the Indo-Pacific region, collected in Madagascar and Mauritius. No significant facts on the nature of their reproductive structures have been published since the pioneering studies of Ascherson[9] and Black[10], reviewed by den Hartog[3]. We have found in both genera that flowering occurs in early summer, and the plants are dioecious with separate male and female individuals. The flowers are hidden within the leaf sheaths. The paired anthers (Fig. 1*a*) extend upwards by peduncle elongation at maturity, when the entire structure is abscinded and floats free at the time of anther dehiscence. The female flowers remain hidden, the filamentous stigma branches elongate when receptive and protrude between the leaf sheaths (Fig. 2*a*).

The pollen grains are remarkable. They are elongate and confervoid, each more than 6 mm long, non-aperturate and at maturity they are coiled like a spring within the anther (Fig. 1*b*). They are trinucleate, with the nuclei located

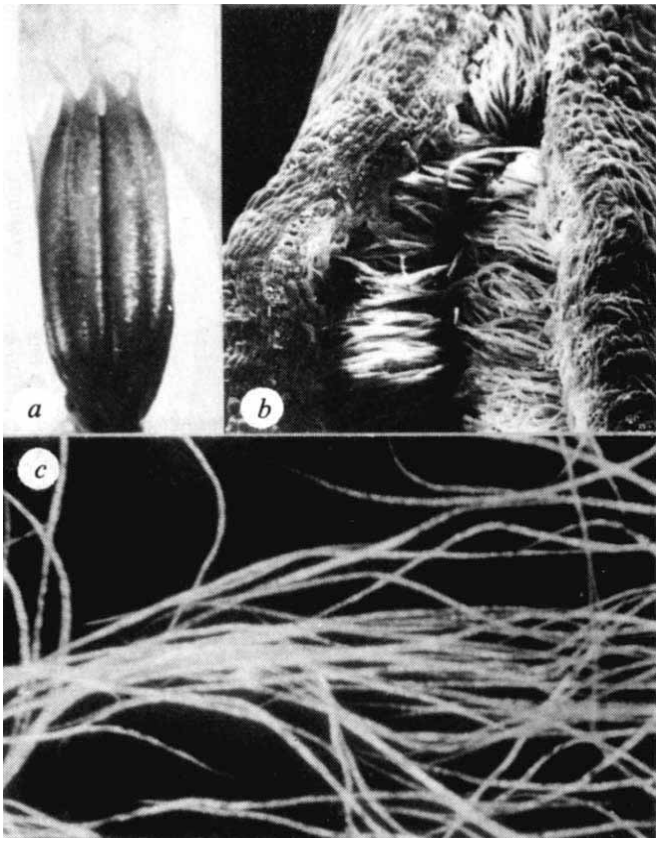

Fig. 1 Male flowers of the sea nymph, *Amphibolis antarctica*. *a*, Male inflorescence consisting of paired anthers fused to form a single dispersal unit (×4). *b*, Scanning electron micrograph of anther freeze dried at dehiscence, showing release of coiled pollen grains (×45). *c*, Vermiform pollen grains dehiscing from anther into seawater, revealed by the fluorochromatic reaction[19], a test for pollen viability. The cytoplasm of the grains shows intense green fluorescence (×80).

centrally. The walls are unusual in apparently having no outer exine, which in terrestrial plants is made of the polymer sporopollenin[11]. Cytochemical tests have revealed no trace of exine. In transverse sections, the walls of mature pollen stain a uniform red colour with toluidine blue, like the inner intine layer of terrestrial angiosperm pollens. Microspectrophotometric analysis of the red stain has revealed only the 565-nm peak characteristic of the intine, and not those of the green-stained exine (590 and 640 nm) of the terrestrial plants; fluorescence microscopy after staining with 0.01% auramine O at *p*H 5.0, which provides even higher resolution, has revealed absence of the yellow fluorescence characteristic of the exine (personal communication from J. Heslop-Harrison). Transmission electron microscopy of other aquatic plants[12] has demonstrated a progressive loss of exine, though in no case was it absent.

The grains adhere together, forming a rope-like structure when teased from a mature anther into seawater (Fig. 1*c*). The pollen is soft and flexuous, bending in water currents as it escapes from the anther. The surface appears smooth under scanning electron microscopy, and a faint reticulate pattern is evident at higher magnification. The tips of the grains grade into a pair of fine, curved hooks, which presumably enable the pollen grains to link together on dehiscence and to attach to the smooth stigma surface. As well as these adaptations to the marine environment, seagrass pollen has some features in common with land plants[13]. Seagrass pollen walls contain cytochemically-detectable acid phosphatase activity, except that the enzyme reaction product is distributed throughout the wall instead of being confined to the inner intine layer. Furthermore, esterase activity, usually restricted in terrestrial plants to the outer exine layer[14], occurs on the surface of the grains in immature anthers associated with the invasive tapetum but is absent in mature pollen.

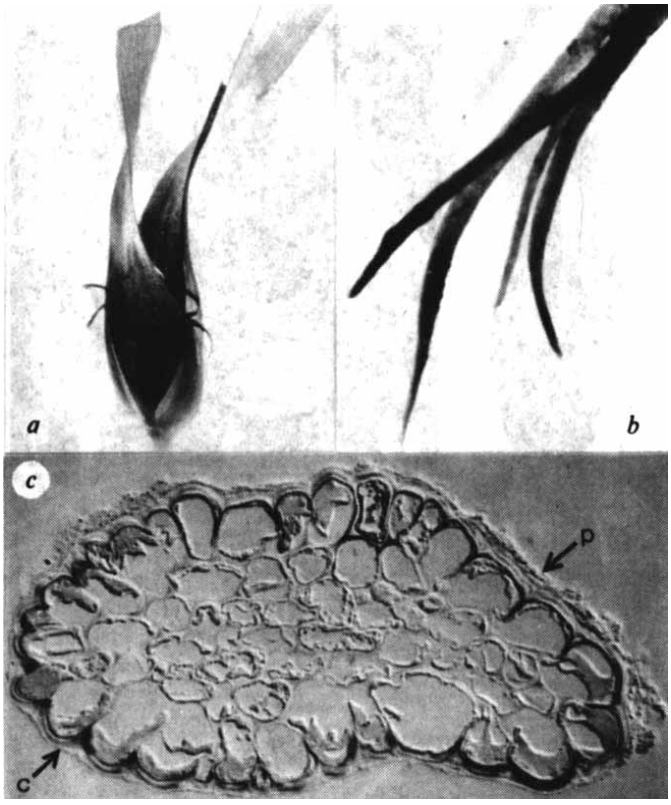

Fig. 2 Female flowers of *Amphibolis antarctica*. *a*, Receptive stigma branches protrude from the leaf-like bracts (×2). *b*, Tips at higher magnification after cytochemical reaction for esterase, with α-naphthyl acetate as substrate in a simultaneous coupling reaction with hexazotised pararosanilin[7,15,16]. The red reaction product appears as a smooth coating on the extreme tips (×12). *c*, Stigma tip treated as (*b*), in transverse section, viewed by Nomarski interference contrast illumination, showing localisation of esterase in papillar (epidermal) cell walls and the outer extracuticular surface (pellicle). *c*, Cuticle. p, pellicle (×350).

In both genera, the paired female flowers each have a style bearing several stigma branches. The tips are coated, when receptive, with a surface layer which can be detected cytochemically by its esterase activity (Fig. 2*b*). Such layers are characteristic of both wet and dry stigmas of land plants, and have been implicated in pollen recognition[15,16]. In terrestrial species, the layer can be partially solubilised in buffer and detergent[5]. In *Amphibolis*, however, the esterase activity of the coating does not disperse readily in seawater. In stigma sections, enzyme reaction product is present in the outer walls of the epidermal cells which correspond to the papillae of terrestrial plants and in the extra-cuticular layer secreted from the cells on the surface (Fig. 2*c*).

Fertile fruits of *Amphibolis* are frequent, so that communication between the pollen released into the sea from male flowers and the stigma does indeed occur. Pollination takes place when the plants are totally submerged, and must involve unique adaptations for cell recognition. The surface of the flexuous pollen grains is adhesive, suggesting the presence of a coating, possibly glycoprotein in nature, that is either resistant to the solubilising action of seawater, or constantly being secreted from within the grains. Arabinogalactan proteins are secreted in large quantities into the medium from *Lolium multiflorum* endosperm cells in liquid culture[17], suggesting that such a mechanism is not without precedent.

Both *Amphibolis* and *Thalassodendron* are viviparous[3] and the single basal embryo develops directly into a plantlet. After fertilisation, special floral structures differentiate to enable the seedling to find a foothold on the sea bed. Vivipary of this kind is also found in other intertidal plants like mangroves and the monocotyledon *Cryptocoryne*[18].

We believe that the features we have described are those of a group of angiosperms that has adapted successfully to the marine environment by the loss or modification of terrestrial features.

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matters arising

Nucleosynthesis and anomalous Xe and Kr in carbonaceous chondrites

BLACK[1] has suggested that the anomalous Xe isotopic composition known as carbonaceous chondrite fission (CCF) Xe results from a varient of the r-process and is not due to fission. To yield the CCF Xe isotopic composition, the r-process peak due to the neutron magic number 82 would need to occur at mass 136 rather than 130 as in standard Solar System material[2].

An actual r-process scenario has two difficulties. First, r-process calculations yield an abundance peak far too steep compared with the CCF data, when the abundance peak occurs close to the magic number on the valley of β stability (for the magic number $N=82$, β stability is at mass 138). Second, the time scale of an r-process resulting in a peak within two mass units of β stability is very long compared with that of an exploding object (the r-process time scale is determined by the β rates, which are relatively long near the valley of β stability); thus there is no plausible astrophysical site.

Another closely related possibility exists: a neutron capture process much faster than an s-process but operating under the same principle of competition between neutron capture and β decay. We have prepared a computer program to carry out such generalised neutron capture (n-process) studies[3]; the neutron capture cross sections required by the program code are derived from the Hauser–Feshbach code of Holmes *et al.*[4]. This n-process may occur in novae, supernovae shocks or in explosive supernovae shells.

We have examined the predicted Xe and Kr relative (heavy) isotopic abundances as a function of neutron flux for neutron temperatures of 2–155 keV (Fig. 1). Also shown in Fig. 1 are the recent Allende experimental results of Anders *et al.*[5,6] and the implied heavy Xe spectrum for Murchison[7] after a large fractionation correction (the non-fractionation corrected Murchison heavy Xe spectrum looks like the Allende heavy Xe spectrum). All other proposed heavy Xe spectra lie somewhere between these two curves. It is not possible to get a good fit for either the Anders *et al.* unfractionated Xe or the Kr data and, furthermore, the best fit for each does not occur for the same flux. These results are for 155-keV neutrons, similar results obtain at other neutron energies down to 2 keV. Near β stability the cross section computer code is good to a factor of order two (S. E. Woosley, personal communication); greater changes in neutron cross sections would be needed and specifically selected to get a good fit to the unfractionated Allende results. Such a selected set of cross section changes is not likely. Note, however, that the heavy Xe spectrum corrected for a large implied fractionation is not very different from a possible n-process. If further experimental work indeed shows the carbonaceous chondrite heavy Xe spectrum to be relatively smooth without a dip at 132, then perhaps the results can be explained with some sort of an n-process. Unfortunately, the Kr results cannot be fitted, but they are probably less reliable than the Xe results (E. Anders, personal communication). If the heavy Kr spectrum of Anders *et al.* is verified then a steady-state n-process explanation is no longer possible.

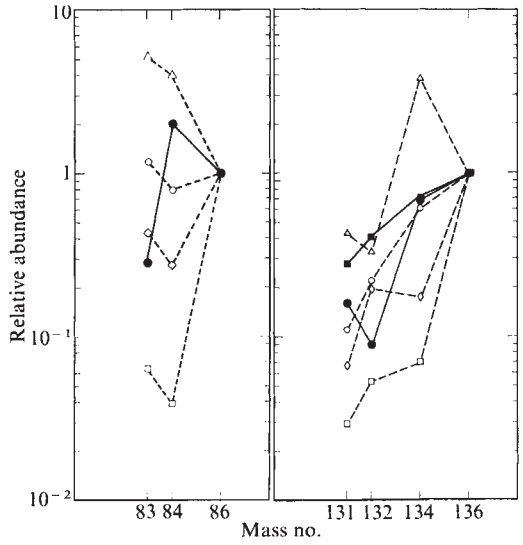

Fig. 1 The calculated isotopic abundances of Kr and Xe as a function of neutron flux. Also shown are Allende experimental results of Lewis *et al.*[6] and the Murchison Xe results of Srinivasan *et al.*[7] with their large fractionation correction. The calculations may fit the Murchison xenon results but they cannot fit the Lewis *et al.*[6] Kr results, nor a heavy Xe spectrum with a large dip at 132. ●, Allende; ■, Murchison; neutron flux on an arbitrary scale: □, 1; ◇, 30; ○, 70; △, 300.

There is another important point with regards to the Allende xenon results of Anders *et al.*[5] and Lewis *et al.*[6]. Pepin[8] has pointed out that enriched light Xe isotopes seem to be a separate component from the heavy isotopes. From examination of the relevant nuclear processes, it seems clear that the light Xe anomalies cannot be fitted with spallation reactions —such reactions always produce $^{126}Xe > ^{124}Xe$ for any plausible seed distribution. Perhaps the explanation of the anomalous light isotopes lies in either the p-process[9] or in fractionation[10]. If the light xenon component is due to the p-process then ^{130}Xe might be effected, which would change the normalisation[11] on the heavy Xe, tending to decrease the dip at 132. Thus both the p-process and fractionation, if consistently applied, will tend to smooth the heavy Xe.

These n-process computations were done using the long time steady-state solution and thus are independent of initial composition. We conclude that a classical r-process cannot yield the observed Xe–Kr isotopic distribution. A long time n-process would fit only if there was no dip in the Xe spectrum at 132 and if the carbonaceous chondrite Kr spectrum were very different from that observed by Anders *et al.* It is also possible however, that the anomalous gas distributions could result from the addition of only a few or several neutrons to already existing heavy seed nuclei. (One can get a good fit if the number of free parameters equals the number of isotopic abundances to be fitted; such a procedure is not very useful.)

We are currently examining the results of adding a few neutrons to pre-existing seed and limiting the free parameters by using plausible theoretical

pre-supernovae isotopic compositions and by investigating what other observable effects would result from such a neutron burst. These may well be limited to the other noble gases; effects on other elements may be masked by their much greater normal abundance. The superheavy element suggestion of Anders *et al.*[5] of course remains a possibility.

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Elevation of selenium levels in air by xerography

THE report on this subject which appeared in *Nature*[1] raises questions of methodology and relevance which I discuss here.

Although the authors calculate airborne selenium concentrations between 2×10^{-8} and 6×10^{-8} g m^{-3}, a simple calculation based on the data presented yields a concentration range 4.9×10^{-9}–14.7×10^{-9} g m^{-3}. Of more concern is the sensitivity of the analytical methods used. Their prime reference[2] reports "precision and accuracy are good for Se levels down to about 0.1 p.p.m. (1×10^{-7} g per determination) and acceptable for many purposes to 0.02 p.p.m. (2×10^{-8} g)" and reagent blanks ranging from 2 to 2.3×10^{-8} g selenium per determination. Using Olson's method[2], Harkin *et al.*[1] reported collection of from 5×10^{-9} to 15×10^{-9} g of airborne selenium in a xerography room. These values are clearly below Olson's own sensitivity limit and are even below Olson's blanks[2]. Harkin has made assurances (personal communication) that Olson's methods have been improved on and that the values reported are in excess of blanks, but the precision and accuracy of Harkin's data cannot be verified by the methods cited.

Even assuming Harkin's results are valid, some perspective is appropriate. The highest selenium concentration reported[1] (6×10^{-8} g m^{-3}) is 3,333 times lower than the US Occupational Safety and Health Agency (OSHA) limit[3] (2×10^{-4} g m^{-3}). If one assumes a human minute volume of 8.2 l (ref. 4) and total absorption of inspired selenium[5] at the highest level reported, the total selenium ingested by inhalation in an 8-h day (2.4×10^{-7} g) would be 27 times less than that ingested by consuming a single 23-g slice of white bread (6.4×10^{-6} g)[6]. Selenium, incidentally, is considered as a nutritionally essential trace element[7].

We have measured selenium emissions from Xerox machines and have found them to be several orders of magnitude below the OSHA limit and below the usable limit of our analytical methods. Since the question has been raised, however, we have initiated a renewed effort externally to ensure that we have the most accurate data possible.

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HARKIN ET AL. REPLY—Fluorimetric analysis of selenium using 2,3-diaminonaphthalene (DAN) is easily sensitive enough to determine the levels we collected from air. The sensitivity claimed[1] for the method is very conservative and can be improved by slight modifications of the basic procedure[2]. These include recrystallisation of the DAN reagent, extraction of the fluorescing Se–DAN complex with cyclohexane rather than with decahydronaphthalene, and extension of the digestion time with perchloric–nitric acid to 90 min following the first appearance of perchloric acid fumes. Sensitivities of 1 ng and <4 ng have been claimed for blank analyses by others[3,4]. Our blank values lay reproducibly at 4.7 ± 0.7 ng Se.

The question[5] of the sensitivity of the method is not really pertinent, since the figures we reported[6] were for levels above values measured in control experiments using air samples collected in laboratories in the same building as our copying room. Because the values were low for single-day air samples, to increase the reliability of the analyses, cumulative samples taken over 4 d were actually analysed, but values were supposed to be expressed on a daily basis. Omission of the word "daily" after the values of 0.005–0.015 ng in the report[6] made them seem to be the 4-d total rather than single-day averages. The value of 20–60 ng Se per m^3 of air is the correct, rounded-off figure for the airborne Se levels.

Tolerable limits for exposure to selenium depends on the Se species concerned, and some reappraisal of current values may be warranted. The time-weighted average concentration of 0.2 mg m^{-3} permitted by the OSHA for elemental selenium and selenium oxides in air differs from the value for hydrogen selenide, which at 0.05 p.p.m. is 200 times lower than that for hydrogen cyanide, which is generally considered to be the most highly toxic common airborne inorganic compound[7]. Russian workers recently suggested that the permissible limits for SeO_2 should be reduced to 0.1 μg m^{-3} in air for a single exposure and to 0.05 μg m^{-3} in air for average daily exposure[8].

Comparison of inhaled and ingested selenium is of doubtful validity in the absence of toxicological work directed at this specific question. There is, in fact, evidence that selenium administered in the oxidised form can be excreted in part by respiration as volatile methylated derivatives[9].

Although excess selenium may be harmful under ordinary circumstances, its beneficial effects in counteracting heavy metal toxicity should not be overlooked[10–13]. Since there are many questions of selenium biochemistry and metabolism still unanswered, the effects of minute volatile emissions from copiers cannot be predicted with confidence. We appreciate the efforts of copier manufacturers to ensure that these emissions will be kept to a minimum and checked by independent analyses.

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reviews

Delights of opisthobranch molluscs

V. Fretter

Biology of Opisthobranch Molluscs. Vol. 1. By T. E. Thompson. Pp. 207. (The Ray Society, c/o British Museum (Natural History): London, 1976.) £15.

OPISTHOBRANCHS, the unexpected progeny of shelled gastropods, have relinquished their protective encasement to reveal a grace of form and colour unsurpassed by other molluscs. Yet most have remained relatively unknown to the malacologist because of their retiring and migratory habits and their fondness for the immediate sublittoral, an area difficult to explore before the days of SCUBA diving. This is the first book related to British opisthobranchs for over a hundred years. It is in two sections: the first, dealing with the general biology of the group, includes some magnificent colour photographs and drawings of external features of worldwide forms; the second is a systematic treatment of British species, excluding nudibranchs (which are to be dealt with in a second volume), the parasitic pyramidellids and the planktonic pteropods.

No comprehensive account of the biological activities of sea slugs has been written previously and in the first section the author successfully conveys his interests in, and personal experience of the living animals. The text is lucid and not loaded with excessive detail so that its contents are easily accessible to the non-specialist. It comprises a series of chapters dealing in a general way with various aspects of opisthobranch biology from the point of view of functional anatomy; these constitute one of the outstanding features of the book.

These aspects are dealt with almost on the 'type' system since each consists largely of summaries of the author's own research on a number of species. Without wishing to detract in any way from the value of this, I wish that the relationship to other opisthobranch research had been explained more fully to provide a better understanding of the biology of the group as a whole, although, as opisthobranchs have radiated so divergently, this may be too difficult a task. I wish, too, that the coverage of the systems had been complete—reluctance to enter the field of neurophysiology, where opisthobranchs have furnished so much important material, is easily understood, but there are other aspects of the nervous system than the physiological; and there are other systems in regard to which the views of so experienced and distinguished a worker would have been welcomed.

The systematic section summarises characters of all orders, provides keys to British species dealt with in this volume and a description of each, including comments on the living animal. This will be an invaluable guide to the taxonomist. He, like others, would be helped by a subject index.

Illustrations of anatomy are from the author's published works, those of co-workers and redrawings of the anatomy of even some of the most accessible species from classical illustrations published as far back as 1845.

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Meditations on a cell cycle

Multiplication and Division in Mammalian Cells. By Renato Baserga. Pp. xii+239. (Dekker: New York and Basel, May 1976.) SFr. 78.

PROFESSOR BASERGA'S book combines those rare qualities of being readable, informative and very often amusing. The book makes no claims to be an exhaustive study of the multiplication of mammalian cells; ample references direct the reader to review articles of greater depth.

A major part of the monograph is naturally taken up with a review of the cell cycle and aficionados of the relevant literature will recognise Baserga's rather personal views on cell cycle controls. Baserga marshalls a number of arguments (none of them convincing) to support the widely held idea that G_0 cells are not merely cells with a long G_1 which may be shortened by suitable stimulae, but are a separate class of cells awaiting their fairy godmother to trigger them back into the cycle. This is not just a semantic argument as it determines whether mitogenic stimulae are regarded as triggers initiating new processes or accelerators of ongoing events. I see nothing wrong with the view that G_1 may be infinitely expandable to include G_0. Unfortunately the cell cycle is often drawn like the face of a clock with the cell cycle phases apparently given definite durations. This ignores the fact that variation in the length of cell cycle phases (especially G_1) is the rule rather than the exception, and failure to recognise this has placed enormous psychological constraints on the understanding of cell proliferation. The criteria Baserga cites for distinguishing between cells in G_1 and cells in G_0 result from sampling cells from widely separate parts of a continuum of proliferation rates.

The other hobby-horse Baserga rides round the cell cycle is the idea that new genes must be activated to trigger cells from quiescence to rapid growth. This may be true but I find his evidence involving changes in the physical structure of chromatin, chromatin template activity and nuclear acidic proteins far from conclusive. Recent mRNA hybridisation studies show that at most only 5% of the approximately 10^4 separate mRNA sequences differ in resting and growing cells. As only 5–10% of the mammalian genome is transcribed this difference represents less than 0.5% of the total genome. I find it difficult to believe that the large scale changes in chromatin with which he makes such a great play) are all devoted to the derepression of such a minute fraction of the genome.

This being said, much of the book is good sense; anyone involved in cell proliferation could do well to read it if only as an antidote to the turgid prose and thought behind much of the burgeoning hard core of the cell proliferation literature. **Robert Shields**

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Monoamine oxidase

Monoamine Oxidase and Its Inhibition. (Ciba Foundation Symposium 39 (new series).) (In Honour of Mary L. C. Bernheim.) Pp. xii+415. (Elsevier/Excerpta Medica/North Holland; Amsterdam, Oxford and New York, 1976.) Dfl.78; $29.95.

THE present resurgence of interest in monoamine oxidase (MAO) and its inhibitors (MAOIs) stems directly from the discovery that at least two functionally distinct forms of the enzyme exist in many mammalian tissues, including the human brain. The problem is now to assess the physiological and pharmacological significance of this finding particularly with regard to the use of MAOIs in the treatment of certain affective disorders. This volume is intended to update and assemble some of the more recent findings in the field.

Almost without exception, the papers comprising this book are of a high standard. The first five articles deal with the nature and properties of the multiple enzyme forms together with the mechanism of action of acetylenic MAOIs. The second section catalogues a series of re-investigations into the physiological significance of MAO using MAOIs that more or less specifically inhibit one or other of the enzyme forms. A final group of seven papers deals with the evaluation of these newer MAOIs in the treatment of certain types of depressive illness and with also the possible usefulness of changes in human blood platelet MAO activity as indicators of such disease states as depression, migraine and schizophrenia.

The most interesting and revealing portions of this volume are the 125 pages of discussion. A feeling of bewilderment and uncertainty is constantly surfacing throughout these pages. Is the 'amine hypothesis' of depressive illness the correct model? Against all preconceptions, may antidepressant drugs act by reducing and not increasing the effective concentration of pharmacologically active amines at the receptor site? Perhaps the site of action of MAOIs is not in the brain but in the periphery: the accumulated amines may then penetrate the blood–brain barrier. Are MAOIs effective antidepressants because they inhibit MAO or is it because they inhibit amine synthesis by a feedback mechanism or perhaps block the uptake of amines by cellular elements in the brain? Is the ability of the tricyclic antidepressant drugs to inhibit one of the two forms of MAO with a K_i of about 10^{-5} M a factor in their mood-elevating properties? Is it even possible to make meaningful studies on the role of MAO *in vivo* when that fraction associated with nerve endings is less than 5% of the total? These and many other problems are discussed in this fascinating volume.

It seems absurd that so little is known about the role of an enzyme originally characterised as long ago as 1928 by Mary Bernheim. There is one inescapable conclusion that may be drawn from this book: until the aetiology of depressive illness is understood and the biochemists and clinical psychiatrists achieve some standardisation in their respective disciplines, the treatment of affective disorders with MAOIs will continue to be a 'hit-and-miss' affair. **Godfrey G. S. Collins**

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Mammalian gluconeogenesis

Gluconeogenesis: Its Regulation in Mammalian Species. Edited by Richard W. Hanson and Myron A. Mehlman. Pp. xxvi+592. (Wiley-Interscience: New York and London, 1976) $36.40; £18.60.

THIS volume of 15 essays is dedicated to Professor Henry A. Lardy for his outstanding contributions to biochemistry–impressively listed in the introduction. The editors have sought to compile the most significant information gleaned from 25 years of research on gluconeogenesis, an area in which Professor Lardy has played an important part. The chapters are grouped into four sections: the enzymology of gluconeogenesis; the involvement of the mitochondrion; the experimental approach to gluconeogenesis in liver and kidney; and gluconeogenesis in man. The sections seem to cover the main areas of current interest: the properties and regulation of the enzymes unique to glucose production that do not function in glycolysis; the vital question of the concentration of substrates actually available to these enzymes; the significance of the intracellular location of the enzymes, particularly phosphoenolpyruvate carboxykinase (PEP-CK); transmitochondrial membrane carriage of reducing equivalents; the interaction and competition for energy and substrate between urea synthesis and gluconeogenesis; the different manner of control of gluconeogenesis in liver and kidney; and last but by no means least what it all means for *homo sapiens*.

As the editors point out we now know a great deal about the pathway of gluconeogenesis from various substrates, but considerably less about how the process is controlled. It remains unclear whether for glucose formation from lactate and alanine pyruvate carboxylase or PEP-CK constitutes the pacesetting enzyme. The activity of the former is strongly regulated by acetyl-CoA, but it is pointed out that we can at present only guess at the concentrations of CoA esters available to the enzyme *in vivo*. PEP-CK exists in at least two forms and possesses a varied distribution between mitochondrion and cytoplasm in different species. The level of the cytoplasmic form in particular responds to a number of hormones. Although the varied distribution is described by several authors and the range of activities in different species compared, there seems no suggestion of what the rationale for this diversity may be. The enigma of an apparent K_m for PEP-CK towards oxaloacetate far in excess of available concentrations, resolved for the cytoplasmic enzyme by Ballard (*Biochem. J.* **120**, 809, 1970), remains an acute problem for the mitochondrial enzyme since the overall mitochondrial concentration of oxaloacetate seems so low. It was Lardy himself who pointed out the virtue of a cytoplasmic location of PEP-CK if it led to oxaloacetate transfer from the mitochondrion as malate, since this simultaneously resulted in 2H transfer to the cytoplasm needed at the stage of triose phosphate formation when gluconeogenesis is proceeding from substrate not initially undergoing oxidation. It is interesting to learn that in pigeon liver, where phosphoenolpyruvate synthesis is almost entirely mitochondrial, negligible glucose synthesis from either pyruvate or alanine occurs. Except in relation to man, little consideration seems to be given to the role of glycerol as a gluconeogenic substrate, yet in prolonged fasting it becomes quantitatively important and could also be a source of reducing equivalents for other precursors.

This volume will be indispensable to all workers in the field and is to be recommended to anyone interested in gluconeogenesis. It is a pity that a number of the chapters have no summary section, but, perhaps to compensate, the editors have provided their own synopsis of the contributors' main points and the current state of the art. **K. L. Manchester**

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obituary

Hermann Träuble was born on April 7, 1932 at Nellingen, near Ulm, and died on July 3, 1976.

It was about nine years ago that Hermann Träuble first put his head round my door with a rhetorical "Am I disturbing you?" This pleasant young man who stood there before me, with a modest and yet forceful presence, was by no means an unknown quantity to me. His teacher and supervisor at the Max-Planck-Institut in Stuttgart, Alfred Seeger, had notified me of his visit, with the words which Mozart is said to have used of Beethoven: "Keep an eye on him, one of these days he'll make the news".

Hermann Träuble had already made the news. Together with Uwe Essmann he had successfully carried out an experiment which others had only dreamed of—the direct, visible demonstration of a quantum effect. This experiment is so typical of its performer's straightforward, goal-directed methods of thought and work that I would like to outline it here in a few words. I shall try to relate the story in the way which Hermann Träuble himself described it to me.

The experiment was an attempt to demonstrate the quantisation of magnetic lines of force in a particular class of superconductors, the so-called Class II superconductors, which are characterised by a particular kind of behaviour in a strong magnetic field.

How does one make the paths of magnetic flux lines visible? The answer is known even to schoolboys: use fine iron filings—but just how fine must the filings be, to demonstrate the quantum phenomenon? One would answer intuitively: "As fine as possible, best of all a dust made up of individual atoms or small aggregates of atoms." But that would be a hasty answer, for we must recall that ferromagnetism is not a property of individual atoms, and it can only appear in ordered crystalline structures.

The question should therefore more precisely run: how small must the particles be, to respond to a quantum effect, and how large must they be to act ferromagnetically and to appear on the screen of an electron microscope.

The two young scientists grasped the bull by the horns, and simply started to experiment. Using an inert-gas atmosphere, they succeeded in producing tiny crystals with an edge length of ~ 0.000001″, and then in condensing these onto the surface of a semiconductor in a magnetic field. The circulating currents in the semiconductor induced by the magnetic field are divided up into tiny vortices, which rotate about an axis parallel to the magnetic lines of flux. The minute ferromagnets deposited themselves preferentially onto this lattice of 'quanta of magnetic flux' and build up crystal nuclei, which could be seen under an electron microscope.

Result: a direct, macroscopic observation of a microscopic quantum effect!

The Physics Prize of the Göttingen Academy of Sciences awarded to these two scientists was a clear expression of the recognition with which the world of learning received this great achievement. For Hermann Träuble this was, however, not the first distinction. A short while previously he had received the Masing Preis of the German Metallurgical Society for his work on the magnetisation and hysteresis in ferromagnetic single crystals, which he had carried out as part of his doctorate.

Now he was standing in front of me. All he wanted was a post as an assistant —a man who had a brilliant career in solid-state physics in front of him and to whom a university chair was already open. All he wanted was a small laboratory, he assured me, maybe a hundred square feet—after taking a critical look at the dimensions of my study. He had taken it into his head to get into biophysics and a true Swabe can never be deflected from his intended course.

Hermann Träuble's interests in Göttingen were particularly directed at the structure and function of lipid membranes. In 1971 he published a comprehensive article in *Die Naturwissenschaften*: 'Phasenumwandlungen in Lipiden', whose subtitle 'Possible Switching Processes in Biological Membranes' already looked to the future. He hitched his own work onto that of Dennis Chapman, who had demonstrated calorimetrically the phase transitions between crystalline and liquid-crystalline states. Hermann pioneered the development of new and above all fluorimetric methods which made it possible to follow these rapid co-operative transformations. Thus he was able to investigate not only the static but also the dynamic aspects of these processes. The high speed of the phase transformations is closely connected with their potential significance as biological switching processes. Collaboration with Hansjörg Eibl made possible a comprehensive study of those membranes whose lipid constituents were already well known. Models were developed, their electrostatic interactions were studied, and the findings compared in each instance with similar or related effects in biological systems.

These were the pioneer years of a new 'membrane biophysics', full of excitement and creativity. Seldom did Hermann go home before daybreak, and even then he was always back in the Institute at five to two in the afternoon—just in time to grab something to eat in the canteen.

Wherever co-operation was offered, it was taken up. Peter Overath contributed his wide experience of the Coli membrane, and through this collaboration a detailed picture of the structure and phase transformations of bacterial membranes was put together, backed up by studies with models. Together with Erich Sackmann, who was familiar with the technique of electron spin resonance, he attacked the problem of lateral diffusion of the lipid molecules in the membrane. This turned out to be a neck-and-neck race with the group of Harden McConnell in Stanford.

He also applied his research to fundamental biological problems—a classic example of this is the paper with Hansjörg Eibl and Hideo Sawada "Respiration—a Critical Phenomenon?"; it is still too early to see the significance of his investigations for the final understanding of nerve membranes. Not without good reason was Hermann Träuble a much sought-after discussion partner in numerous workshops in the Neurosciences Research Program in Boston, USA. Listening to his lectures was a pure delight. His vital asset was that, where others had only answers, he always posed the right question.

The walls of his original laboratory had long since become too narrow. In 1974 the Max-Planck-Gesellschaft received him as a Scientific Member, and only a few weeks before his death he was appointed to a directorship in the Institute for Biophysical Chemistry in Göttingen. His research group had grown uninterruptedly in recent years, and includes today a dozen scientists, who will undoubtedly go on to extend the ideas which they and Träuble developed.

Neither was there any lack of new honours. The Faculty of Human Medicine of the University of Giessen

awarded Hermann Träuble the Ludwig Schunk Prize in 1972 and the German Bunsen Society for Physical Chemistry the Bodenstein Prize in 1975.

The man, just 44, was called away at the zenith of his career. We shall not meet Herman Träuble again in the corridors of the Institute, or be enlivened at our tea colloquia by his burning enthusiasm and swabish charme; we shall not race together down the ski slopes in January, and I shall never have another chance of digging with him for fossils in California. Certainly, death is an integral part of life. But this insight cannot take the pain away.

Manfred Eigen

Ian Macpherson, head of the Department of Virology at the Imperial Cancer Research Fund Laboratories, London, died on September 11, 1976 at the age of 46, in the middle of a distinguished career. He was married with three children.

Macpherson was born and educated in Scotland, as a Foundation Scholar at George Herriots, and after at Edinburgh University, leading to a Carnegie Fellowship and a Ph.D. in virology in 1955. He then worked at the Microbiological Research Establishment, Porton, for four years before moving to Glasgow in 1959. After a year in the Department of Genetics, he became a founder member of the new Institute of Virology and of the Medical Research Council's Experimental Virus Research Unit. Except for a year as an Eleanor Roosevelt Cancer Fellow at the Wistar Institute, Philadelphia, he remained in Glasgow until 1968 when he was appointed to the senior staff of the Imperial Cancer Research Fund Laboratories in London to head a substantial department.

Macpherson began to study tumour viruses in Glasgow and it was for his unique and pioneering contributions to the study of virus transformed cells that he will be remembered. Of great importance was the isolation of the first line of cultured cells (BHK 21 cells) which could be used for precise quantitative evaluation of the transformation process, and which allowed direct comparison of transformed and untransformed cells from the same clonal population. Then came the discovery, with Montagnier, of the agar suspension assay system, which, with its various modifications, is of profound importance in tumour cell biology. Macpherson also isolated the first revertants of transformed cells, thus paving the way for the use by others of this important method for studying the transformed cell phenotype. In recent years Macpherson and his colleagues in London concentrated on the altered surface chemistry of transformed cells, and in particular developed the use of conditional mutants for elucidating the role of the virus.

Through Macpherson's wide reputation, his laboratory attracted visitors from far and near. He lead his group from the laboratory bench, not only by his originality and knowledge but by his experimental ability. Those associated with him soon discovered another aspect of his character as well—his impish sense of humour. This was often expressed in remarkable collages in which he delighted to ridicule any pompous and unjustified aggrandisment.

Ian Macpherson's hobby was golf, in which he also excelled, and it was on the golf course that he died.

The eminent organic chemist **George O. Curme, Jr** died on July 28 at his summer home at Oaks Bluff, Massachusetts. He was born in Mount Vernon, Iowa in 1888, the son of a professor of German who was also a renowned grammarian. Curme graduated at Northwestern University, then as a graduate student moved from Harvard to the University of Chicago, where he obtained his Ph.D. Next he went to Germany, then the Mecca of organic chemists (it has been said that in the first half of the century all organic laboratories were built pointing towards Berlin) to study under Fritz Haber and Emil Fischer at the Kaiser Wilhelm Institute and the University of Berlin.

On returning to America he worked from 1914–1920 as a research fellow at the Mellon Institute, and it was here that he started on the work which founded the synthetic alipathic organic chemistry industry in America. (Aliphatic chemistry deals with chain-like compounds of carbon, as opposed to aromatic chemistry which deals with ring compounds.) He initially sought a method for the commercial manufacture of acetylene; he later widened his horizons to many other alipathic compounds.

In 1917 he started to work for Union Carbide, with whom he was to remain (eventually becoming a director) until his retirement. From the Union Carbide laboratories in 1925 he announced the discovery of a process to make ethylene glycol, which soon replaced alcohol for use as an antifreeze in cars' radiators, and which rapidly developed into a multi-million dollar industry. Dr Curme continued his research, producing a new synthesis of ethyl alcohol, and later playing an important part in the wartime synthetic rubber programme.

Among the many awards he was given was the Willard Gibbs Medal, bestowed on him by the American Chemical Society in 1944 for his fundamental role in bringing "leadership in organic chemistry from Germany to the U.S.A."

announcements

Meetings

November 17–19, **ACTH and Related Peptides: Structure, Regulation and Action,** New York (Mr Charles Roarty, The Barbizon-Plaza Hotel, 106 Central Park South, New York, New York 10019).

Winter Gordon Research Conferences:
January 3–7, **Deformation and Failure Mechanics in Polymer Composites; Bacterial Cell Surfaces.**
January 10–14, **Polymers; Organic Thin Films and Solid Surfaces.**
January 17–21, **Electrochemistry; Agricultural Science.**
January 24–28, **Chemical Oceanography.**
January 31–February 4, **Hormone Action.**
(Alexander M. Cruickshank, Director Gordon Research Conferences, Pastore Chemical Laboratory, University of Rhode Island, Kingston, Rhode Island 02881).

June 1–3, **Frequency Control,** Atlantic City, New Jersey (Deadline for abstracts: January 21) (Commander, US Army Electronics Command, ATTN: DRSEL-TL-MF (Dr J. R. Vig), Fort Monmouth, New Jersey 07703).

September 5–9, **Precise Electrical Measurements,** Brighton, Sussex (Conference Secretary, The IEE, Savoy Place, London WC2R 0BL).

September 5–10, **Geochronology, Cosmochronology and Isotope Geology,** Pisa, Italy (Deadline for abstracts: May 15) (Gabriella Bonadonna, C.N.R.-Laboratorio di Geochronologia, Via Cardinale Maffi, 36, 56100 Pisa, Italy).